Microbiological Qualities of Some Foods Sold in the Street and in the Mid-level and High-level Restaurants

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DECLARATION

This is to declare that the research work embodying the results reported in this thesis entitled "**Microbiological Qualities of Some Foods Sold in the Street and in the Mid-level and High-level Restaurants**" submitted by Asma Binte Afzal, has been carried out under the joint supervision and able guidance of Professor Dr. Naiyyum Choudhury, Coordinator, Biotechnology and Microbiology Program, and Associate Professor Dr. M. Mahboob Hossain , Microbiology Program, BRAC University in partial fulfillment of MS in Biotechnology, at BRAC University, Dhaka. It is further declared that the research work presented here is original, has not been submitted anywhere else for any degree or diploma.

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Dedication

TO

My Beloved

Parents

Acknowledgement

At the outset, I would like to express my sincerest appreciation to the Almighty for the continuous blessings that provided me the strength and perseverance to be able to carry out this research study positively and complete this dissertation.

This research study and dissertation appears to be in this current form because of great support and assistance from several people. I would like to thank all of them. Firstly, I would like to express deepest gratitude and much appreciation to my supervisor Professor Naiyyum Choudhury for the continuous support and kind assistance. I would like to extend my sincere thanks to my co-supervisor Dr. Mahboob Hossain (Associate Professor) for his kind cooperation throughout the research study. My heartfelt thanks to Md. Fazle Rabbi, senior lecturer, Microbiology program, for his valuable opinions and ideas throughout the study. I would like to thanks Professor A. A. Ziauddin Ahmad, Chairperson, Department of Mathematics and Natural Sciences, BRAC University, for the treasured counsels, assistance and inspiration in this study.

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Abstract

Street foods and restaurant foods play an important role in people's daily food options as well as their regular nutritional requirements are dependent on these foods, as their ever-growing busy schedule take away the opportunity to eat homemade food. Due to expedient availability, these street and restaurant foods are one of the primary food options for city people. Over the years, many food-borne diseases have been reported due to contaminated non-homemade food consumption. This study was conducted to analyze the microbiological quality of foods which are sold in street side, mid-level restaurants and high-level restaurants. This research study observed , and analyzed the microbiological quality of seven most commonly consumed food items of street side carts, mid-level restaurants and high-level restaurants of Paribagh and Kawran Bazaar areas of Dhaka city, Bangladesh. Total viable count (TVC), Coliform and Enteric pathogen count (CEC), *Staphylococci* count (SC), Fungal count (FC) and also the presence of urinary tract infection causing pathogens, *Salmonella* spp., *Shigella* spp. were observed in this study. It has been found out that, cooked food items showed less microbial load than raw food. Mid-level restaurant foods showed less microbial load than street side cart and high-level restaurant foods. According to the biochemical test results 27% invasive pathogens, 61% opportunistic pathogens and 12% rare pathogens were found in food samples. Invasive food pathogens were *Staphylococcus aureus* (54.54%), *Bacillus cereus* (27.27%), *Shigella dysentriae* (9.09%), *Proteus mirabilis* (9.09%) and also positively identified some opportunistic and rare pathogens from obtained food samples. This study specifically highlights the level of microbial loads found in various available non-homemade foods. Finally, this study recommends some preventive measures which the government and food-maker together should follow, and also maintain the standard hygienic procedure to prepare, cook and handle foods. Implementation of such measures, rules and regulations on street food vendors and restaurant foods are extremely crucial to maintain the hygienic condition as well as to avoid such spreading of harmful organisms through consumption of contaminated foods.

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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

TVC: Total viable count

CEC: Coliform and Enteric pathogen

SC: Staphylococci Count

FC: Fungal Count

NA: Nutrient Agar

Mac: MacConkey agar

MSA: Mannitol Salt Agar

EMB: Eosine Methylene Blue Agar

SDA: Sabouraoud Dextrose Agar

XLD: Xylose lysine deoxycholate agar

TCBS: Thiosulfate-citrate-bile salts-sucrose agar

Chapter 1

Introduction

1.1.Introduction

People are more attracted to non-home made foods like, restaurant foods and street foods. Non-home made foods are cooked and presented in such a way that fascinates the people. It is not always about the fascination it is about the time to prepare food to eat, e.g. lack of time people of all age from student to job holder have to rely on non-home made foods. Usually non-home made foods are not hygienic, to keep the attention of customer and earn more money with less effort vendors compromise with food quality. People suffer a lot due to consumption of unhygienic foods.

In this study restaurants are classified according to food pricing, menu styling; high-end or high level restaurant's food price is high, menu is different from mid-level or mid-end restaurants. In high-level restaurants variety of food items are available than mid-level restaurants. In mid-level restaurants fixed and limited items are cooked whether in high-level restaurants different meal packages are offered including buffet system where minimum 40 food items are included in the menu. Different parties and functions are also organized in high-level restaurants. Each table or customer gets assigned stewards to serve specific table. In mid end restaurants no such facilities are available; people come to eat regular meal and stewards are limited in number. Usually hygiene level is not strictly maintained as high level restaurants. In developing countries low to mid income earner are higher than high income earner, so basically customer load is higher in mid-level restaurants as good quality foods are cheaper than high end restaurants. Foods are freshly prepared time to time to serve the customer. In this study microbial quality of mid level restaurant's foods are better than high-level and street side stall's food. The microbial quality of high-level restaurant foods is better than street foods. Foods are defined as ready-to-eat foods and beverages prepared at home or street and consumed at street without further preparation (S. Rane, 2011). Low income population of developing countries meet their nutritional requirements by consuming affordable street foods which have unique and convincing flavor (Ackah*et al.*, 2011; Cross *et al*., 2007; Muzaffar *et al.*, 2009). The street foods are usually sold by vendors or hawkers at street side on cart or in a street side small stall. Street vendors are usually poor, uneducated, lack of knowledge of food hygiene, handling, sanitation, environment, food service, hand washing, source of raw materials and potable water (Bhowmik, 2010). Microbial food borne illness is major health problem associated with street foods (Biswas*et al*., 2010;

Tabashsum*et al*., 2013; Mamun*et al.,* 2012). Multidrug resistant food borne microorganisms made the food safety situation more vulnerable in public health (Ali *et al.,* 2011). Diarrhea is most food bone common disease and approximately 30 million people are suffering from food borne illness each year in Bangladesh (FAO, 2012). Microbial toxin or human body's reactions to the microbe cause the disease (Khairuzzaman*et al.*, 2014).

The current study has been done to observe the microbial load of non-home foods from street and restaurants. Seven food items were selected such as, rice, dal, ruti, cake, biscuit, laddu and sugarcane juice; these food items were collected from street, mid-end and high-end restaurants of Paribagh and Kawran bazaar to observe the microbial quality of foods. These food items were selected on the basis of daily consumption of people. Rice is staple food of Bangladesh and the alternative of is rice is ruti. Dal is used to consume with rice and ruti both. Rice, ruti and dal are heavy food items; people consume these foods in sufficient amount that fill the stomach. However, cake, biscuit and laddu are light foods, people often eat these foods whenever feel hungry. Sugarcane juice is common cooling drinks for people mostly available at street side. This sweet sugarcane drink is popular among people, whenever they become tired and thirsty they drink sugarcane juice. Beside this sugarcane juice aloevera juice is also popular among people. Both this juices are good for health; however aloevera juice have medicinal properties. Paribagh is commercial and resident area; however Kawran bazaar is one of the large whole sale market place of Dhaka. Foods were collected aseptically and analyzed in BRAC university microbiology lab. Total viable count, enteric pathogen and coli-form count, *Staphylococci* count and fungi-yeast count were observed. Beside these counts selective media were used to observe the presence of urinary tract infection causing pathogens, *vibrio* spp., *salmonella* and *shigella*. This was done for food quality analysis.

Seven selected food items' microbial quality was analyzed from street side stores, mid-level restaurants and high-level restaurants. This type of study was conducted first in Bangladesh. After observing microbial growth and biochemical tests, the test results were put into ABIS online software which has shown four most probable bacteria name. According to this result further analysis were done. Pathogens were categorized into three groups such as, Invasive, Opportunistic and Rare pathogen. Each invasive and opportunistic pathogen was biochemically analyzed; cause of food contamination and the diseases they cause were also discussed.

SPSS IBM 20 software was used for statistical analysis of quantitative results their correlations with collection places and food qualities. Correlations and regression between water activity of food items and their microbial load, T-test was performed for analysis of means of total viable count of high-level restaurant foods of Paribagh and Kawran bazaar, ANOVA (Analysis of Variance) test was performed for variability of means of total viable count of street side store, mid-level restaurant and high-level restaurant foods.

1.2. Literature Review

Different studies were performed to analyze the microbial quality of street food and homemade food and some studies on street food, homemade and restaurant foods. This study was conducted to analyze the microbial quality of street, mid-level and high-level restaurant foods.

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

Ha ThiAnh 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 are not always safe for consumption compared to homemade and restaurant foods, reported by **Kampen** in 1998 in Jakarta. Some scientists reported comparison of homemade and street food items. In 2012 **Odu NN and Akano UM** reported that homemade 'Shwarma' (Middle Eastern Arabis style sandwich) composed of mixed meats of lamb, chicken, turkey, beef and goat with bread, has shown less total viable count than street ones from three different places of Port Hurcourt Nigeria, due to poor sanitation in street shwarma preparation. Another food item 'Panipuri' (snacks) composed of pani (sour tarmarind soup), puri (crispy fried flour ball), masala (spices) was collected from street side cart of different areas and also homemade ones to compare the enteric microorganisms and microbial load. This study was carried out by **D. G. Solankiand N. R. Dave**in Rajkot, India in 2011 and they also reported that homemade ones showed less microbial load due to proper hygienic preparation of panipuri.In2014, **Nazin.P** and**Jaganathan. A** have been reported that multiple food items from street of Salem district of Tamil Nadu, India showed more viable microbial count (spores, yeast, Gram –ve rod and Gram +ve cocci) than same homemade food items, due to unhygienic food preparation and storage at appropriate temperatures, exposure to flies, dust, wind and other contaminants. Another study was done by **Nkere*et al.***in 2011, which compare three different methods of coliform count of food items collected from street vendors and restaurants. This study was mainly focused on comparison of three different coliform count methods of foods, not on microbial quality analysis between street and restaurant foods.

1.2.2. Microbial analysis of street food

Ready-to-eat foods or street foods are prepared and sold in street side or other public places by vendors (**Artemis P. *et al* 2000, Redmond, E.C. *et al.,* 2003**). Street food consumption is rapidly growing phenomenon in urbanized countries. High unemployment, low salaries and work opportunities, less social programs are common in developing countries and poor countries and street food consumption also high in these types of countries, as these types of foods are cheap, tasty, and easily available. Mainly people from low socioeconomic bracket and students are most popular consumers of street foods (**Oladipo IC, 2010, Chauliac*et al,* 1998**). Lack of basic infrastructure and services and uncontrollable spreading street food vending operations cause major public health risk. Low educational qualifications, socioeconomic status, lack of knowledge of safe food handling, vendor's mobility, diversity, temporary nature also contribute to public health risk (**WHO 1996, Artemis P. *et al*., 2000**). Street foods are prepared and stored in dirty environment, near contaminated sources and most of the time street foods are not covered and exposed to flies and dust which cause food borne diseases due to consumption (**WHO, 2001, 2003; Muinde and Kuria, 2005; Ghosh*et al.*, 2007, Rane S, 2011**). Insects and rodents carry pathogens from garbage, wastewater, and overloaded drain during their nutrient intake (**Tambekar*et al.*, 2009**). Contamination of street foods persists through preparation and cooking due to the quality of raw materials (**Rane S., 2011**).Lack of potable water for various activities vendors re-use the water for cleaning utensils and used dishes; this has been reported in various continents like Asia, Africa and South America (**Rane S., 2011**). *Staphylococcus spp.* contamination of utensils often occur at vending site due to touch the food after touching the dish washing cloths and water after dish washing and hand washing, which indicates cross contamination of dish water, food preparation surfaces and the street foods (**Mensah P, *et al*., 2002**).

1.2.3. Microbial quality of street food in Dhaka city

In Bangladesh street food vending is distinctive and prevailing part of large informal sector. Different items like snacks such as cake, biscuit, nuts, shingara, nuts and phuchka/chotpoti; whole meals like ruti-bhaji, rice, khichuri-tehari are sold in street side carts. Dhaka city filled with small street side carts for street food vending, as street side food vending requires simple skills, small amount of capital, less facilities, small size. This is the easiest way to income money

in rapid urbanization of Dhaka city (**Muzaffar, *et al.*, 2009**). It has been also reported by **Faruque** and his colleagues that about 200000 street food vendors are working in Dhaka city, as many workers choose the street vending as profession. It has been also reported that street food vendors are mostly available around educational institutions like school, college and university, as a result large number of students consume street vended foods. In developing countries most of the street food vendors have less general perception of food safety issues, inadequate knowledge on proper food handling and their role in the transmission of pathogens due lack of formal education or have few years of schooling (**FAO, 2007, Mensah P, *et al*., 2002**). Students and working people are not only attracted to the street foods also they consume different restaurant foods as well. It has been also seen that sometime the microbial quality of standard restaurant foods is poor than local restaurants, a related study undertaken in Benin city in Nigeria the microbial load of ready-to-eat rice in standard fast food centers is higher than the local fast food centers (**Wogu, 2011**). In Gauteng, South Africa a survey was conducted among street food vendors and most of them maintained a high standard hygiene during food preparation and serving corroborated by the microbial count results (**Martins and Com, 2006**). Restaurant food borne outbreaks were also reported in many countries, large outbreaks in 2005, 2011 reported by different media (**Medscape**).

1.2.4. Advanced Bacterial Identification Software

Bacterial identification online software are used for large number of sample containing study, one of them is ABIS (Advanced Bacterial Identification Software) online software has been used in many research purposes (A. Laukova*et al*., 2013, M. Mahiuddin*et al*., 2014, N. Thangaraj*et al*., 2013, Tim Kahlke*et al*., 2012, Duggirala Srinivas M *et al*., 2013). Bacterial identification on the basis of biochemical tests and further explanations, antibiogram result interpretation, serological test result interpretations are available (**Stoica Costin. *Regnum Prokaryotae*, http://www.tgw1916.net, accessed on Mar 2012**).

1.3. Objectives of the study

1.3.1 General objective:

Food borne illness is common phenomenon in Bangladesh even more or less every country's common phenomenon where general hygienic precautions are not followed. In this study some food items were selected which are analyzed in street side carts, mid-level restaurants and high-level restaurants. Mainly two areas were selected to analyze the foods. Some random foods from street-side were analyzed for microbial load determination. ABIS (Analysis of Bacterial Identification Software) online software reliability also observed by input the biochemical test result.

1.3.2 Specific objective:

- Analyze the microbial load of foods which are sold in street side, mid-level restaurants and high-level restaurants.
- Isolate and identify the pathogenic microorganisms from food; rare, opportunistic and significant pathogens.
- Statistical analysis of microbial load among street-side, mid-level and high-level restaurants.
- Statistical analysis between two area's microbial load foods.
- Analyze the type of pathogenic microorganisms based on their severity of diseases they cause.

Chapter 2

Materials and Method

2.1 Materials

2.1.1 Samples

Total seven types of food samples from street, mid-level and high-level restaurants were collected and the sample number is seventy four. Different random food samples from streets of different areas were also collected and tested; total seventeen food samples.

Table 2.1: Area of sampling, number and types of samples

Area of sampling	Food Item	Sample number				Description of Food Items
		Street	Mid-level restaurant	High-level restaurant	Total	
Paribagh	Rice	3	3	3	9	Rice: Boiled white plain rice
	Dal	3	3	3	9	Dal: Prepared by boiled lentil with onion, green chili, red chili, salt, turmeric, oil, dried leaf
	Ruti	3	3	3	9	Ruti: Dough of flour flattened and cooked in pan
	Cake	3	3	3	9	Cake: Prepared with egg, flour, sugar, oil, essence for aroma, baking powder and baked.
	Biscuit	3	3	3	9	Biscuit: Flour mixed with egg, oil, and sugar then baked.
	Laddu	3	3	3	9	Laddu: Flour of lentil liquefied and fried in small granules and soaked in sugar syrup and a round shape is given by pressing those soaked granules.
	Sugarcane juice	3		3	6	Juice extracted from sugarcane by a machine and freshly served.
Kawran Bazar	Rice	1	1	1	3	
	Dal	1	1	1	3	
	Cake			2	2	
	Biscuit			2	2	
	Laddu	1			1	
	Sugarcane juice	2		1	3	
Total= 74						

2.1.2 Media

Different types of media were used for total viable count (TVC),enteric pathogen andcoliform count (ECC), *Staphylococcus sp*. count and total fungi count. Medias arethe Nutrient agar, MacConkey agar, Mannitol Salt Agar and Sabouraud Dextrose Agar respectively.

Media for bacterial isolation: the culture media used are following,

a) Nutrient agar medium:

It is a common microbiological growth medium used for growth of non-fastidious bacteria. This media used for total bacterial count.

Nutrient agar typically contains 0.5% peptone, 0.3% beef extract/yeast extract, 1.5% agar, 0.5% NaCl, 97.2% distilled water.

b) MacConkey agar medium:

It is a differential and low selectivity medium designed to grow Gram negative bacteria and also distinguish between lactose fermenting (e.g. *Klebsiella* and *Escherichia coli*) from non-fermenting bacteria (*Pseudomonas aeruginosa, Salmonella* species and *Proteus mirabilis*)(Oxoid 1998). It contains bile salts (inhibit the growth of most Gram positive bacteria, except *Enterococcus* and some species of *staphylococcus* i.e. *Staphylococcus aureus*), crystal violate dye (inhibits certain Gram-positive bacteria), neutral red dye (stains lactose fermenting microbes), lactose and peptone. Incubation time will be 24-48hrs at 37^{o}C. *E.coli* produces pink colonies.

MacConkey agar contains Proteose peptone 3g, latose 10g, Bile salts 1.5g, sodium chloride 5g, Neutral red 0.03g, Agar 13.5g, water add to make 1liter and pH adjusted to 7.1±0.2

c) Eosin-Methylene Blue (EMB):

This media used for *E.coli* isolation after 24-48hrs incubation at 37^{o}C and produces green metallic sheen.

d) Mannitol salt agar medium:

It is a differential and selective media for *Staphylococcus aureus*. *Staphylococcus aureus* ferment lactose produce yellow colonies in medium after 37^{o}C for 48hrs incubation.

e) Sabouraud Dextrose Agar:

Specific for fungal growth at 25-30^{o}C and after 3-4 days of incubation white, off-white, pink ,smooth, irregular and mucoid colonies are observed.

f) XLD agar medium:

This medium is specific for *Salmonella* and *Shigella* and incubation period has to be 24-48hrs at 37^{o}C (Feng et al., 2007)

g) TCBS agar medium:

It is a selective media for isolation of *Vibrio* species. This media contains lithium chloride and tellurite. Incubation at 37^{o}C for 24-48hrs *Vibrio cholerae* produces yellow convex smooth colony and other species of *Vibrio* produce green small colonies.

h) HiCrome Agar:

This agar media is selective for urine infection causing microorganisms such as *Klebsiella pneumonia, Enterococcus fecalis, Staphylococcus aureus, Proteus mirabilis, E.coli, Pseudomonas aeruginosa*and produce distinctive different colors on media after 24-48 hrs of incubation.

2.1.2.1 Enrichment broth:

a) Alkaline Peptone Water

It is an enrichment broth used for enrich the food sample to enrich the growth of *Vibrio spp.*if any *Vibrio* spp. present in food sample. The pH of the broth is higher than usual broth which is 8.6±0.2.

b) Selenite broth

This broth helps to enrich the growth of *Salmonella* spp. and *Shigella* spp. if any of these microorganisms present in the food sample. The broth is not autoclavable as selenite activity is destroyed by autoclave temperature.

2.1.2.2 Stock culture media:

Tryptone soya broth **G**lucose **G**lycerol (TGG)

This is a broth for stock culture of microorganisms. One liter broth requires 30gm tryptone soya broth, 5gm glucose and 100ml glycerol.

Skim milk–**T**ryptone soya broth–**G**lucose–**G**lycerol (STGG)

It is another broth for stock the isolates or microorganisms. The recipe is same as previous stock culture broth but this broth additionally requires 20gm skim milk in one liter.

2.1.2.3 Media for biochemical test:

- **MR-VP (Methyl Red- Voges Proskauer)broth**

  It is mixed acid fermentation test in which bacteria breakdown glucose and lower the pH othe medium below 5.0. inoculation of isolates into MR-VP broth and after an incubation period methyl red indicator added to the culture broth and pinkish circle will produced which indicate positive result.

- **VP broth**

  Some bacteria are capable of producing acetoin (acetyl methyl carbinol), a precursor in synthesis of 2,3-butanediol. A pink color will be developed after addition of 40% KOH and a 5% solution of alpha-naphthol into the media if acetoin is produced.

- **Simmons' Citrate Agar**

  Utilization of citrate by microorganisms is observed by changing in simmons' citrate agar after inoculation and incubation of 24hrs.

- **Triple Sugar Iron Agar**

  TSI agar made with slant and butt. Utilization of three sugars such as sucrose, dextrose and lactose and hydrogen sulfide production and gas production is observed in this media. Hydrogen sulfide production is detected by blackening of media due to the presence of thiosulfate and ferrous sulfate in the media. Carbohydrate utilization pattern of microorganisms will determine the enterobacteriaceae and other Gram-negative intestinal-bacilli. Three types of results are observed in incubated inoculated TSI agar

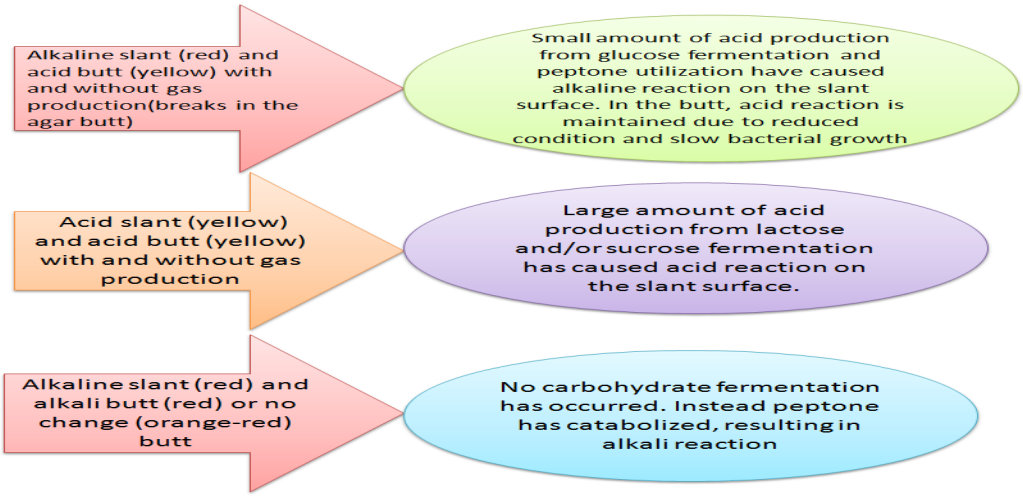

Figure-2.1: TSI agar result interpretation diagram.

- **Motility Indole Urease Agar**

  Urease enzyme activity, indole production and motility of microorganisms are observed in this media.

- **Nitrate Broth**

  Nitrate reduction activity is observed in this broth; where change in broth color after addition of nitrate test reagents detects the presence of nitrate utilization enzyme.

- **Phenol red dextrose broth**

  Utilization of dextrose is observed in this broth, where pH change in media cause color change of the broth.

- **Phenol red lactose broth**

  Utilization of lactose is observed in this broth, where pH change in media cause color change of the broth.

- **Phenol red sucrose broth**

  Utilization of sucrose is observed in this broth, where pH change in media cause color change of the broth.

- **Some specific biochemical test media**

- **Skim milk agar**

  Caseinase (proteinase) activity can be identified by this agar test.

- **Egg yolk Agar**

  Lecithinase enzyme of bacteria breakdown lecithin in egg yolk on egg yolk agar.

- **Starch Agar**

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

- **Blood Agar**

  Lysis of blood cells by microorganisms is observed in blood agar, alpha hemolysis, beta hemolysis and gamma hemolysis are types blood cell lysis pattern by microorganisms.

- **Gelatin Agar**

  Gelatin is another protein some microorganisms are capable lyses gelatin by gelatinase enzyme, which create a clear zone or liquid gelatin form semisolid gelatin after incubation.

2.2. Methods

2.2.1 Sample collection:

Sample collected in sterile tip packet for solid and semi solid food and in bottle for liquid food item from market and stored in refrigerator if it is not possible to sample spreading within 1-2 hour. Figure 3.2, 3.3 and 3.4 show the image of rice sample from three different places.

Figure 2.2: Rice of high-level restaurant

Figure 2.3: Rice of mid-level restaurant

Figure 2.4: Rice of street side stall.

2.2.2 Sample preparation:

Solid and semi-solid foods were blended in blender, before blending blender sterilized for 15-20min with boiled water. Some solid food blended by adding 10gm food item with 90ml 0.9% saline solution and some semi-solid foods were simply blended without adding saline solution. After blending 1gm of sample measured aseptically in three different sterilized small beakers and then 9ml saline (0.9%) solution in one beaker, alkaline peptone water 9ml for *vibrio* enrichment in second beaker and 9ml selenite enrichment broth for *salmonella* and *shigella* culture were added. Alkaline phosphate water and selenite broth enriched for 4-6 hours.

2.2.3 Plating of sample:

Saline solution with sample was serially diluted for six times. For each dilution from 10^{-3} to 10^{-6} plated on duplicate plates of each agar media such as, in Nutrient agar, MacConkey agar, Eosin Methylene-Blue agar, Mannitol Salt Agar and Sabouraud Dextrose Agar respectively. After 6 hours of incubation alkaline peptone water enriched 10^{-1} dilution streaked on TCBS agar media and selenite broth enriched sample streaked on XLD agar media. In HiCrome media 10^{-2} dilution for dry or semi dry food sample and 10^{-4}-10^{-5} dilution for liquid cooked and raw food samples were plated. Chromogenic agar media used for identification of different microorganisms based on their colony color.

2.2.4 Observation and reading:

Growths of microbes are observed after 24 hour incubation and count the colony forming unit in each type of agar plate for specific dilution except the TCBS agar media and XLD agar media, in which presence of specific microorganisms are observed according to the microbial growth pattern. *Vibrio* species give yellow and green colony however on XLD agar *Salmonella* gives black centered pink colony, *Shigella* gives pink colony and *E.coli* gives yellow colony. However in chromogenic agar different microorganisms give different colors such as, *Enterococcus faecalis;* blue, small, *Escherichia coli;*pink-purple, *Klebsiella pneumonia;*blue-purple mucoid, *Pseudomonas aeruginosa;* colorless, *Proteus mirabilis;*light brown, *Staphylococcus aureus;* golden yellow colonies respectively. Microbial growth also observed and noted after 48 hour incubation, then different colonies from different selective media were subcultured on specific media on the basis of colony morphology.

2.2.5 Preservation and Biochemical test:

After 24hour incubation of subcultured colony morphology observed and further subculture on nutrient agar for preservation in tryptone soya broth stock (TGG and STGG) culture media. Gram staining, oxidase, catalase and other biochemical tests such as triple sugar iron test, motility indole urease test, simmons' citrate test, nitrate test, methyl red and Voges-Proskauer test, carbohydrate (sucrose, dextrose, lactose)utilization tests were performed for each type of colony on nutrient agar. 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 on different category of bacteria such as *Enterobacteriaceae*, *Pseudomonas*, *Pasteurellaceae*, *Vibrio* and *Aeromonas*, *Compylobacter* and category of gram positive bacteria are *Streptococcus, Staphylococcus, Clostridium, Bacillus* and *Paenibacillus*. After putting the biochemical test result the software gives 4 most probable bacteria name which accurately match the test results. If detail option is applied after these probable results then it will suggest which test is positive/negative for these four bacteria among the biochemical tests which were performed and also suggest more biochemical tests for specification of bacteria species.

2.2.5.1. Biochemical tests

Before conducting biochemical tests Gram staining has been done,

- **Gram staining**

Bacterial colony smeared on glass slide and sequentially crystal violate, Grams iodine, absolute ethanol and safranin were added then air dried and observed under microscope to differentiate bacteria into large groups; Gram-positive (Fig-2.5,b) and Gram-negative (Fig-2.5,a), based on their cell wall structure.

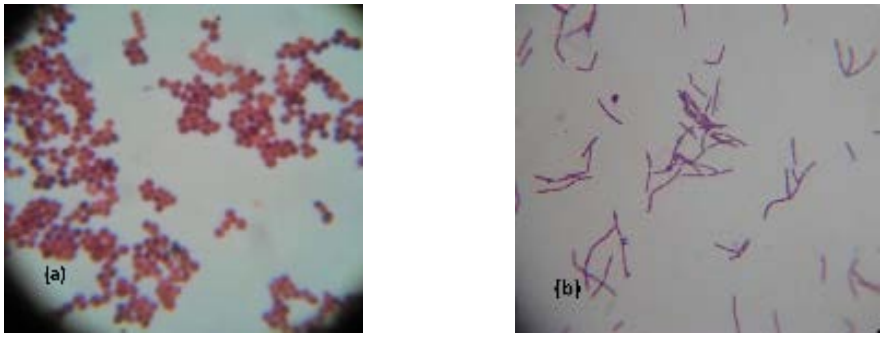

Figure 2.5: Gram negative cocci (a) and Gram positive rod (b)

- **Oxidase**

1) A few drops of oxidase reagent (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) were added onto the Whatman filter paper to soak.
2) A loop full of bacteria from the nutrient agar plate was placed onto the soaked filter paper using sterile tooth pick.

3) Positive reactions turned the bacteria from violet to purple (Fig-2.6) within 1 to 30 seconds. Delayed reactions should be and was ignored.

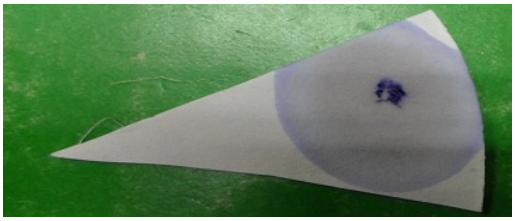

Figure 2.6: Oxidase test.

- **Catalase**

1) A drop of the catalase reagent (Hydrogen Peroxide) was placed on the glass slide.

2) A small amount of bacterial colony was transferred from the nutrient agar plate to the hydrogen peroxide drop using a sterile loop and mixed.

3) A positive result gave a rapid evolution of oxygen within 5-10 seconds and was evidenced by bubbling reaction (Fig-2.7).

4) A negative result showed no bubbles.

Figure 2.7: Catalase test.

- **Triple Sugar Iron 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, CO2 and H2S production.

3) A yellow (acidic) color in the butt indicated that the organism being tested capable

of fermenting all the three sugars (Fig-2.8),

Figure 2.8: Triple sugar iron test.

whereas red (alkaline) color in the slant and butt indicated that the organism being tested is a non fermenter.

4) Detection of H2S 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.

- **Motility Indole Urease**

1) The test was carried out in motility indole urea semisolid medium.

2) One suspected isolated colony was collected with a straight wire and stabbed carefully

into down the tubes without touching the bottom.

3) Following overnight 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.

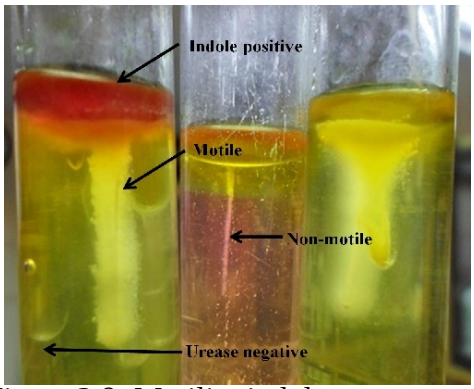

Figure 2.9: Motility indole urease test.

4) Pink color appeared if urea utilized by urease enzyme.

5) Production of cherry red reagent layer (Fig-2.9) after addition of Kovac's reagent in MIU medium demonstrates that the substrate tryptophan has been was hydrolyzed which indicates indole positive reaction

- **Nitrate Reduction Test**

1) Nitrate broth was inoculated with an isolate from each sample plates and incubated for 48hours.

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 (Fig-2.10).

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.

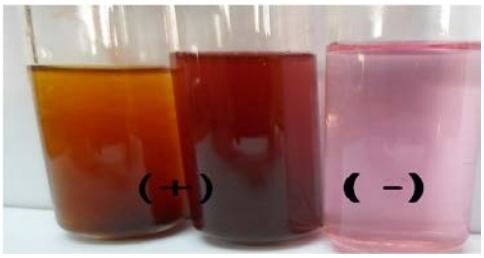

Figure 2.10: Nitrate test

- **Simmons Citrate 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 Prussian blue (Fig-2.11); a negative slant would have no growth of bacteria and would remain green.

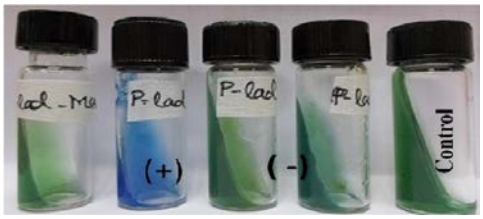

Figure 2.11: Simmons' citrate test.

- **Carbohydrate utilization 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 (Fig-2.12) indicating apositive result.

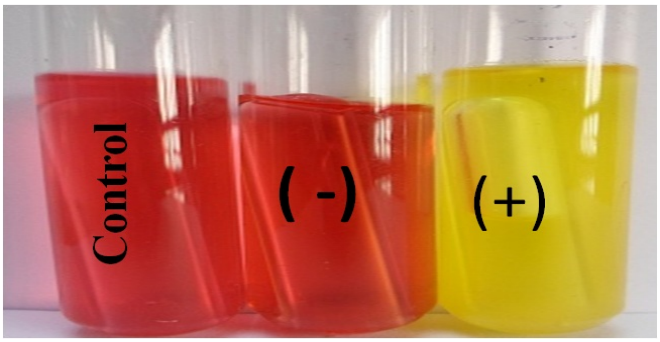

Figure 2.12: Carbohydrate utilization test

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.

- **Methyl Red and Voges-Proscaure 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) Half of this incubated broth transferred into another test tube for Voges-Proscaure Test

4) 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 orange color.

5) Barritt's reagent A was added to the test broth and shaken.

6) Barrit's reagent B was added and the tube was allowed to stand for 15 min (Fig 2.13).

7) Appearance of red color was taken as a positive test and no color development taken as negative.

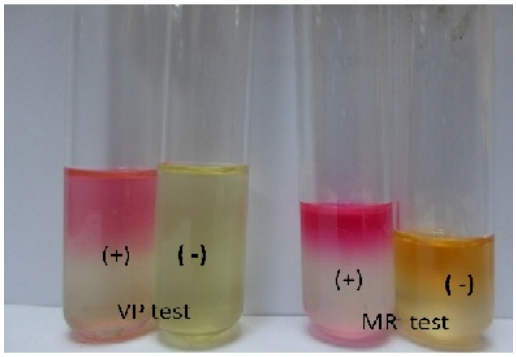

Figure 2.13: Methyl-red and Voges-Proscauer test.

- **Starch hydrolysis test**

1) Specific isolate colonies streaked on starch agar.

2) Alpha amylase enzyme producing strain will produce a clear zone after overnight incubation.

3) The iodine solution was added to the culture medium a hallo zone around the colony zone is clearly observed (Fig-2.14).

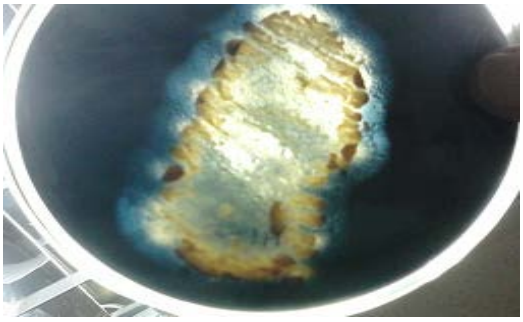

Figure 2.14: Starch hydrolysis test.

- **Lecithinase test**

1) Specific bacteria colony streaked on egg yolk agar.
2) After overnight incubation phopholipase C positive strain broken the phospholipid lecithin (phosphatidylcholine) and an opaque zone (Fig-2.15) has been created.

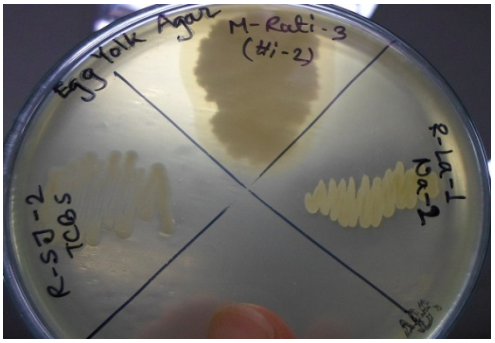

Figure 2.15: Lecithinase test.

- **Hemolysis test**

1) Specific bacteria colony streaked on sheep blood agar.
2) After overnight incubation a clear zone appeared after beta hemolysis, greenish clear zone appeared if the bacteria broke blood cells partially which means alpha hemolysis

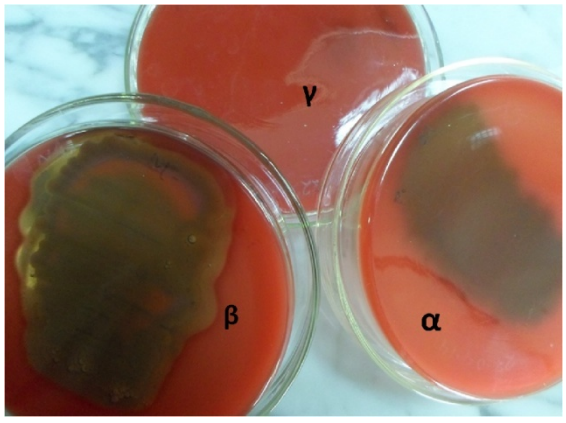

Figure 2.16: Blood hemolysis test

and no clear zone will appeared (Fig-2.16) if the bacteria not able to break down blood cells which indicating gamma hemolysis.

2.2.6. Bacteria Identification Software: ABIS (Analysis of Bacteria Identification Software) online software

Some software are developed which are alternative of commercial systems, code-books or identification tables for identification of microorganisms. Among them ABIS (Analysis of Bacterial Identification Software) online software is popular and effective. This software gives probable bacteria name based on their morpho-biochemical characters, 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.

Table- 2.2: ABIS online software's bacteria taxon categories

Gram positive taxons	**Gram negative taxons**
• *Staphylococcus* • *Streptococcus* • *Clostridium* • *Bacillus and Paenibacillus* • *Lactobacillus*	• *Enterobacteriaceae* • *Pseudomonas* • *Pasteurellaceae* • *Vibrio, Aeromonas* • *Campylobacteraceae*

- ✓ Type ABIS online software in Google search bar
- ✓ Select ABIS online bacterial identification option
- ✓ An web page will appear
- ✓ Insert an user name and choose biochemical test
- ✓ Then choose the respective taxons and then insert the biochemical test results and press continue button
- ✓ Probable four organisms name will appear but the probability and accuracy will be 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 linked with encyclopedia. By clicking this option an elaborate description will be appeared for specific bacteria.

2.2.7. Antibiogram test

1) 2-3 colonies of specific bacteria were collected by loop from fresh subcultured media and inoculated into 0.9% saline solution and mixed well by vortex mixture.
2) The turbidity of the saline solution compared with standard MacFarlane solution.
3) A cotton swab was dipped into the turbid saline solution and made lawn on Mueller Hinton agar (MHA).
4) Specific antibiotic disks were placed on the inoculated MHA 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 MHA according to the susceptibility and resistance to specific antibiotics around antibiotic disks.
6) Clear zone indicates susceptibility (Fig-2.17) of bacteria to the specific antibiotic and no clear zone indicates resistance to the antibiotic.

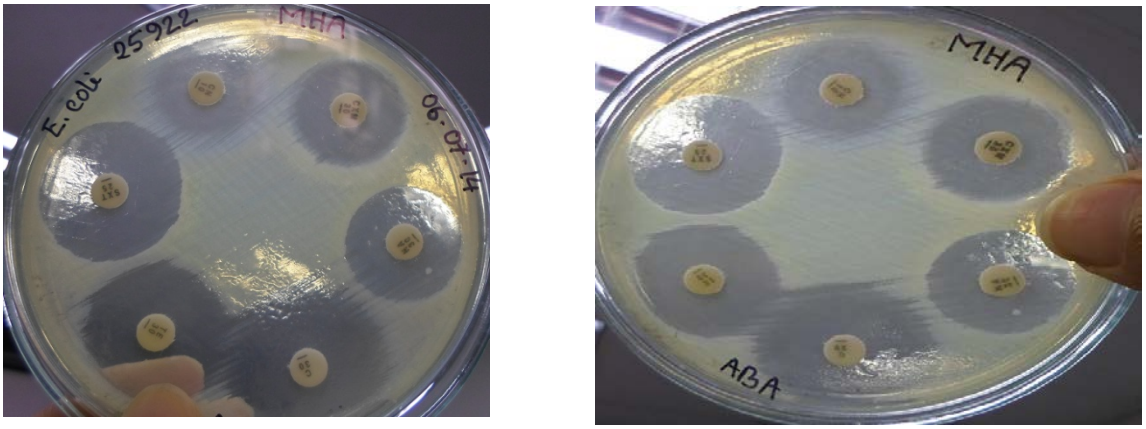

Figure 2.17: Antibiotic susceptibility test.

Chapter 3

Result

3.1. Microbial loads of different food items

This study was based on statistical analysis of microbial loads of different food items of street side, mid-level restaurants and high-level restaurants. The work has been done in two selected area of Dhaka, such as, Paribagh and Kawran Bazar.

3.1.1. Qualitative Result

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

3.1.2. Quantitative Result:

Some selective media were used to observe the presence of some specific microorganisms; TCBS agar for *Vibrio* spp., XLD for *Slmonella* spp. and *Shigella* spp. and HiCrome agar for the bacteria which are responsible for urine infection; different bacteria exhibit different color. The qualitative result is also presented in next sections.

Quantitative Result

Table 3.1- Microbial load of street, mid-level and high-level restaurant foods

Name of area		**STREET** CFU/ml					**MID-LEVEL RESTAURANT** CFU/ml					**HIGH-LEVEL RESTAURANT** CFU/ml				
	Food item	NA	Mac	EMB	MSA	SDA	NA	Mac	EMB	MSA	SDA	NA	Mac	EMB	MSA	SDA
Paribagh	Rice	$3x10^5$	$9.2x10^5$	$2.8x10^5$	$1.5x10^5$	$2.2x10^5$	$5.5x10^5$	$1x10^5$	$3.5x10^5$	$1x10^5$	$3.5x10^5$	$13.8x10^6$	$6.4x10^6$	$19.3x10^6$	$0.1x10^6$	$10.0x10^6$
	Dal	$1.8x10^6$	$0.58x10^6$	$0.74x10^6$	$0.04x10^6$	$132x10^6$	$2.5x10^5$	$0.5x10^5$	$0.1x10^5$	$0.4x10^5$	$3.6x10^5$	$21.8x10^7$	$5.6x10^7$	$14.2x10^7$	$3.7x10^7$	$15.4x10^7$
	Ruti	$7.6x10^2$	NG	NG	$1x10^3$	NG	$0.5x10^3$	NG	NG	$0.2x10^3$	$2x10^3$	$1.11x10^4$	NG	NG	$1x10^4$	$1x10^2$
	Cake	$4x10^5$	NG	$2.7x10^5$	$3.2x10^5$	$7x10^5$	$2.1x10^6$	NG	$0.1x10^6$	$0.09x10^6$	$0.07x10^6$	$4.1x10^6$	$1x10^3$	$1.2x10^4$	$6.2x10^4$	$5.2x10^4$
	Biscuit	$4x10^4$	NG	$0.001x10^6$	$0.1x10^4$	$1x10^3$	$6x10^2$	NG	$3x10^2$	$20x10^2$	$1x10^2$	$3x10^2$	NG	NG	$5x10^2$	$2x10^2$
	Laddu	$2.4x10^4$	NG	$12x10^4$	$0.66x10^4$	$1.2x10^4$	$1.35x10^6$	NG	$0.16x10^6$	$2.27x10^6$	$2.63x10^6$	$1x10^4$	$0.1x10^4$	$0.3x10^4$	$1.4x10^4$	$2x10^4$
	Sugarcane juice	$2.48x10^7$	$1.6x10^7$	$1.91x10^7$	$0.003x10^7$	$5.2x10^7$						$6.93x10^6$	$1.9x10^6$	$4.5x10^6$	$0.4x10^6$	$27.6x10^6$
		STREET CFU/ml					**MID-LEVEL RESTAURANT** CFU/ml					**HIGH-LEVEL RESTAURANT** CFU/ml				
		NA	Mac	EMB	MSA	SDA	NA	Mac	EMB	MSA	SDA	NA	Mac	EMB	MSA	SDA
Karwan Bazar	Rice	$5.5x10^5$	NG	$3x10^5$	$29x10^5$	$3.5x10^5$	$1.38x10^6$	$1.07x10^6$	$1.52x10^6$	$6x10^4$	$3.8x10^5$	$5.2x10^5$	NG	$1x10^3$	$1.03x10^5$	$2.96x10^5$
	Dal	$1.3x10^6$	$0.2x10^6$	$1.75x10^6$	$0.1x10^6$	$1.3x10^6$	$2.88x10^6$	$2.59x10^6$	$2.66x10^6$	NG	$1x10^6$	$4.3x10^7$	NG	NG	$5x10^6$	$4x10^6$
	Ruti															
	Cake											$1x10^6$	NG	NG	NG	NG
	Biscuit											$1x10^5$	$1x10^4$	$2x10^4$	$1x10^4$	NG
	Laddu	$3.0x10^6$	NG	NG	NG	NG										
	Sugarcane juice	$1.5x10^6$	$0.87x10^6$	$1.08x10^6$	$0.002x10^6$	-						$42x10^6$	$26x10^6$	$27x10^6$	$9.5x10^6$	$1.5x10^6$

NA=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar

Quantitative Result

Table 3.2- Presence of various types of microorganisms in different fooditems.

Name of Area		Street			Mid-Level Restaurant			High-Level Restaurant		
PARIBAGH	**Food Item**	**HiChrome**	**TCBS**	**XLD**	**HiChrome**	**TCBS**	**XLD**	**HiChrome**	**TCBS**	**XLD**
	Rice	*Enterococcus fecalis, Staphylococcus aureus, Pseudomonas aeruginosa, E.coli*	Yellow, green	Yellow	Off-white, *E.coli, Enterococcus fecalis, Klebsiella pneumonia*	Yellow, green colonies	yellow	*Klebsiella pneumonia, Enterococcus fecalis, Staphylococcus aureus, Proteus mirabilis*	Yellow, green	Yellow
	Dal	*Enterococcus fecalis, E.coli*, off-white	Yellow, greenish yellow	Yellow black center	*Enterococcus fecalis, Pseudomonas aeruginosa*	yellow	Grayish yellow	*Klebsiella pneumonia, Enterococcus fecalis, Staphylococcus aureus*, off-white	Yellow black center, green	yellow
	Ruti	*Klebsiella pneumonia, Enterococcus fecalis, Staphylococcus aureus* off-white,	yellow	NG	*Enterococcus fecalis, Pseudomonas aeruginosa*	NG	NG	*Enterococcus fecalis, E.coli*	NG	NG
	Cake	*Enterococcus fecalis, Pseudomonas aeruginosa, E.coli*	Off-white, yellow	Deep pink,	Black fungi, *Enterococcus fecalis, Pseudomonas aeruginosa* ,off-white	Yellow, greenish yellow	Pink	*Enterococcus fecalis, Proteus mirabilis*	Yellow, grayish rough	NG
	Biscuit	*E.coli, Enterococcus fecalis, Proteus mirabilis* off-white	Grayish yellow colony	NG	*Staphylococcus aureus, Enterococcus fecalis, Pseudomonas aeruginos*	NG	yellow	*Enterococcus fecalis, E.coli, Staphylococcus aureus*	Green small	NG
	Laddu	*Staphylococcus aureus, Enterococcus fecalis, Pseudomonas aeruginosa, Klebsiella pneumonia, E.coli*	Yellow irr. medium	NG	*Klebsiella pneumonia, Enterococcus fecalis*	Yellow	Grayish centered yellow	NG	Irr.ylw	Yellow clns
	Sugarcane juice	*Enterococcus fecalis, E.coli, Klebsiella pneumonia*	Yellow, greenish yellow	Grayish yellow	*Enterococcus fecalis, , Klebsiella pneumonia, E.coli, Proteus mirabilis* off-white	Black, yellow, deep green				

Blue-green= *Enterococcus fecalis*, purple to magenta= *E.coli*, Bule to purple= *Klebsiella pneumonia*, colorless or greenish pigment= *Pseudomonas aeruginosa*, golden yellow= *Staphylococcus aureus* ,light brown= *Proteus mirabilis*

Qualitative Result

Table 3.3- Presence of various types of microorganisms in different items of food.

Name of Area		**STREET**			**Mid-Level Restaurant**			**High-Level Restaurant**		
K ARWAN BAZAR	**Food Item**	**HiChrome**	**TCBS**	**XLD**	**HiChrome**	**TCBS**	**XLD**	**HiChrome**	**TCBS**	**XLD**
	Rice	Not Available	Yellow	Yellow	*Klebsiella pneumonia*, *Pseudomonas aeruginosa* cream	Yellow, green	Yellow, pinkish yellow	*Pseudomonas aeruginosa*, *E.coli*, *Enterococcus fecalis*	Greenish yellow	NG
	Dal	Not Available	Yellow, green, black	Yellow black	*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *E.coli*, cream	Yellow, green, black	Yellow black	*Pseudomonas aeruginosa*, off-white	yellow	Yellow
	Cake							NG	NG	NG
	Biscuit							NG	NG	NG
	Laddu		NG	NG						
	Sugarcane juice							*E.coli*, *Enterococcus fecalis*, *Klebsiella pneumonia*	Yellow	Yellow

Blue-green= *Enterococcus fecalis*, purple to magenta= *E.coli*, Bule to purple= *Klebsiella pneumonia*, colorless or greenish pigment= *Pseudomonas aeruginosa*, golden yellow= *Staphylococcus aureus*, light brown= *Proteus mirabi*

Qualitative Result for HiCrome Media

Presence of different types of bacteria in different food items grown on HiCrome media

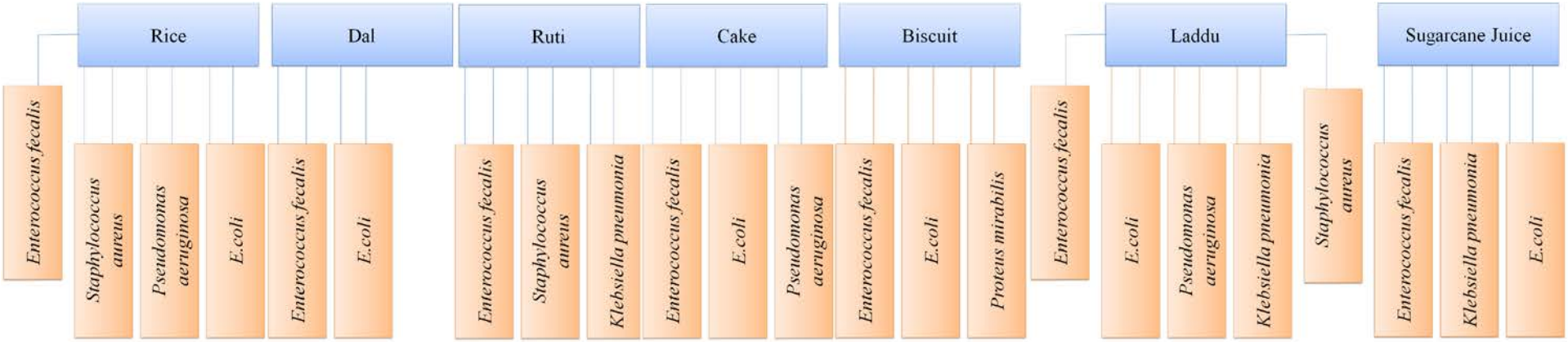

Figure 3.1: Name of bacteria in selected street foods according to HiCrome media result.

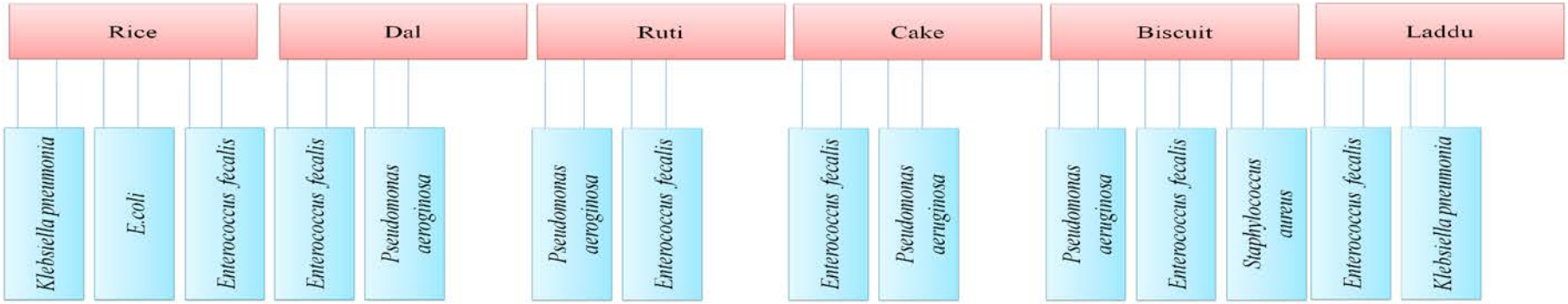

Figure 3.2: Name of bacteria in selected mid-level restaurant foods according to HiCrome media result

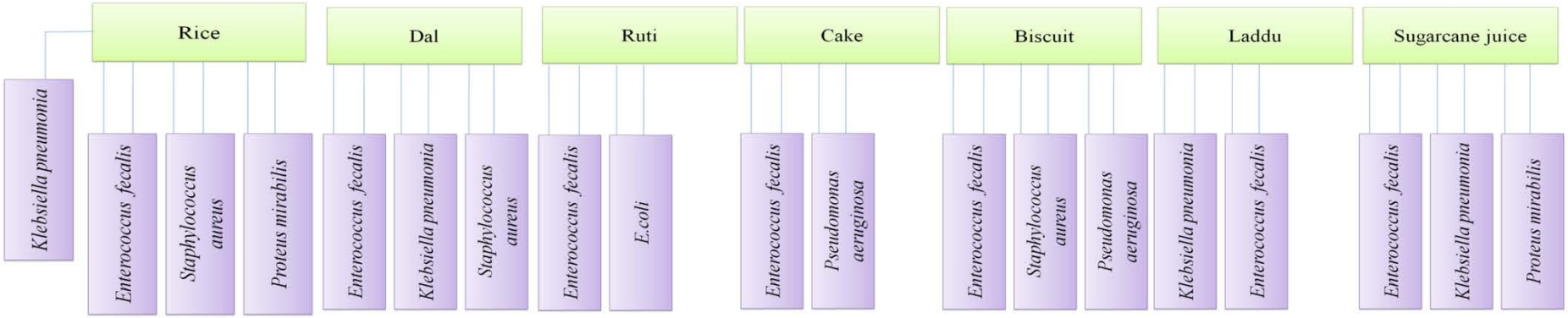

Figure 3.3: Name of bacteria in selected high-level restaurant foods according to HiCrome media

3.2. Microbial load of street, mid-level and high-level restaurant's food items of Paribagh area

3.2.1. Microbial load of street foods

Microbial loads of food from different places vary because of various reasons. In this study microbial load of street, mid-level and high-level restaurant foods are different. The variation of microbial loads in different foods are graphically presented below,

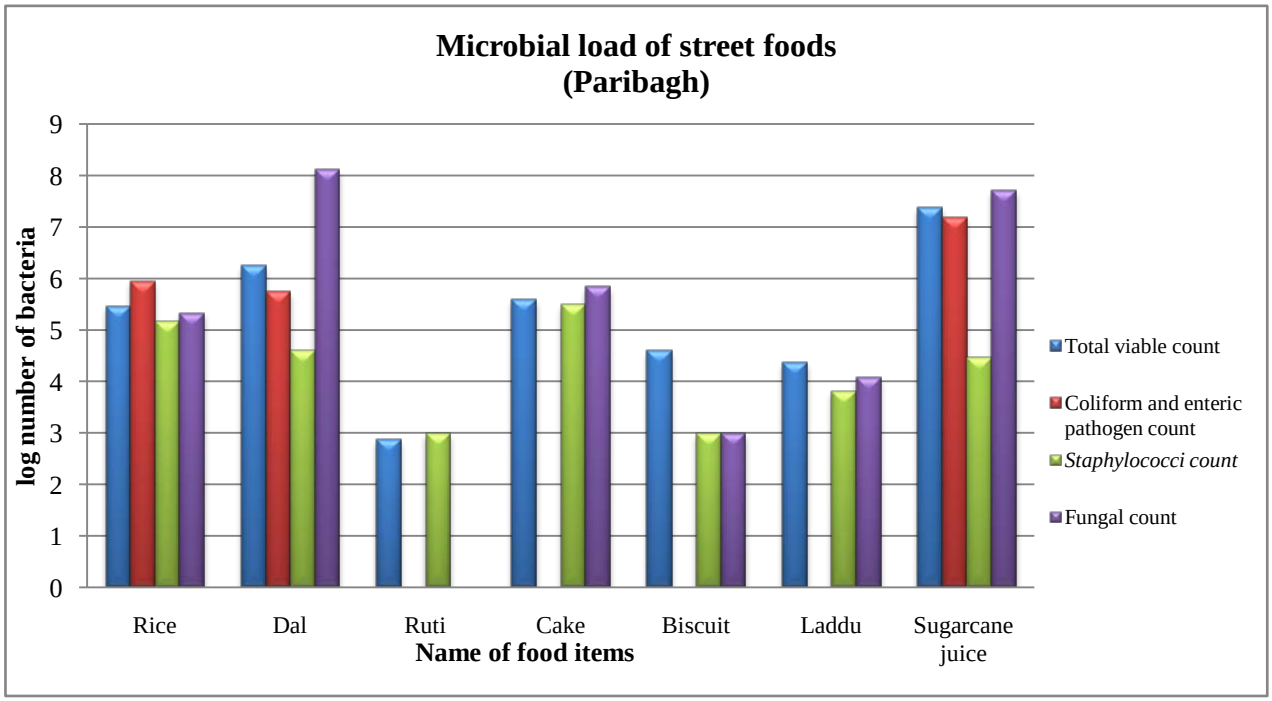

Figure-3.4: Microbial load of street food of Paribagh.

From Fig.3.4, it can be stated that rice, dal and sugarcane juice gave four types bacterial count results, which are total count, coli form and enteric pathogen count, *staphylococci* count and fungal count. However other food items gave only three or two types of bacterial count. Cake, biscuit and laddu have shown total viable count ($4x10^5$,$4x10^4$, $2.4x10^4$), *staphylococci* count ($3.2x10^5$, $3x10^3$, $6.6x10^3$) and fungal count ($7x10^5$, $3x10^3$, $1.2x10^4$). Total viable count ($7.6x10^2$) and *Staphylococci* count ($3x10^3$) were also observed in ruti.

Microbial count was converted into logarithmic scale to give a proper chart format. High fungal count was observed in dal ($5.2x10^7$) and sugarcane juice ($1.32x10^8$). Among all foods sugarcane juice has shown highest total viable count $2.4x10^4$, coliform and enteric pathogen count $1.6x10^7$and lowest total viable count, *staphylococci* count and fungal count observed in ruti and biscuit.

3.2.2. Microbial load of mid-level restaurant foods

Same food items were collected and plated to observe microbial growth which was previously done for street side stall's food items.

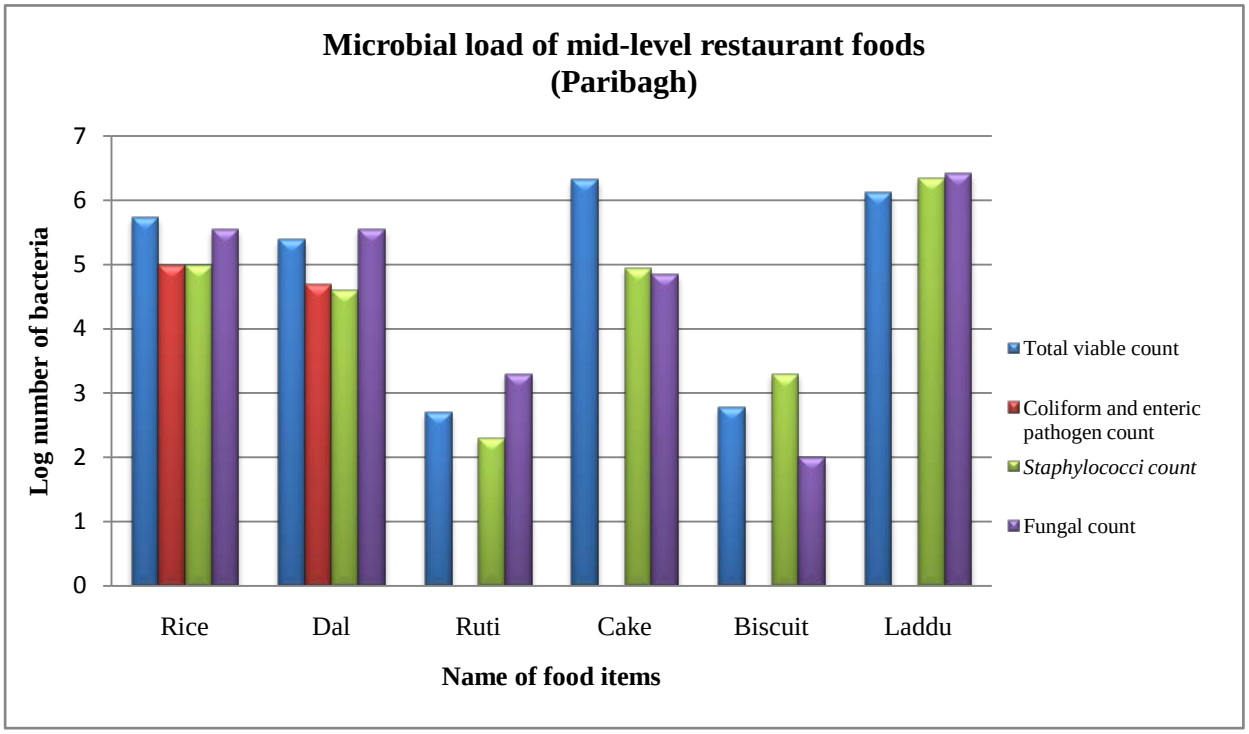

Figure-3.5: Microbial load of Mid-level restaurant food of Paribagh.

Total four types microbial count were observed in rice and dal. However ruti, cake, biscuit and laddu have shown three types of bacterial count; total viable count ($2.1x10^6$, $6x10^2$, $1.35x10^6$), *staphylococci* count ($9x10^4$, $2x10^3$, $2.27x10^6$) and fungal count ($7x10^4$, $1x10^2$, $2.6x10^6$) (Fig-3.5). No coli-form and enteric pathogen count were observed in those food items. Lowest total viable count was observed in ruti, biscuit then dal, rice, laddu, cake in a descending order. In ruti lowest *staphylococci* count ($2x10^2$) was observed and highest ($2.27x10^6$) in laddu. In case of fungal growthladdu has shown the highest count and then other food items such as, rice, dal, cake, ruti and biscuit respectively.

3.2.3. Microbial load of high-level restaurant foods

The microbial growth of same food items which were previously observed for street and mid-level restaurant foods were also observed for high-level restaurant foods.

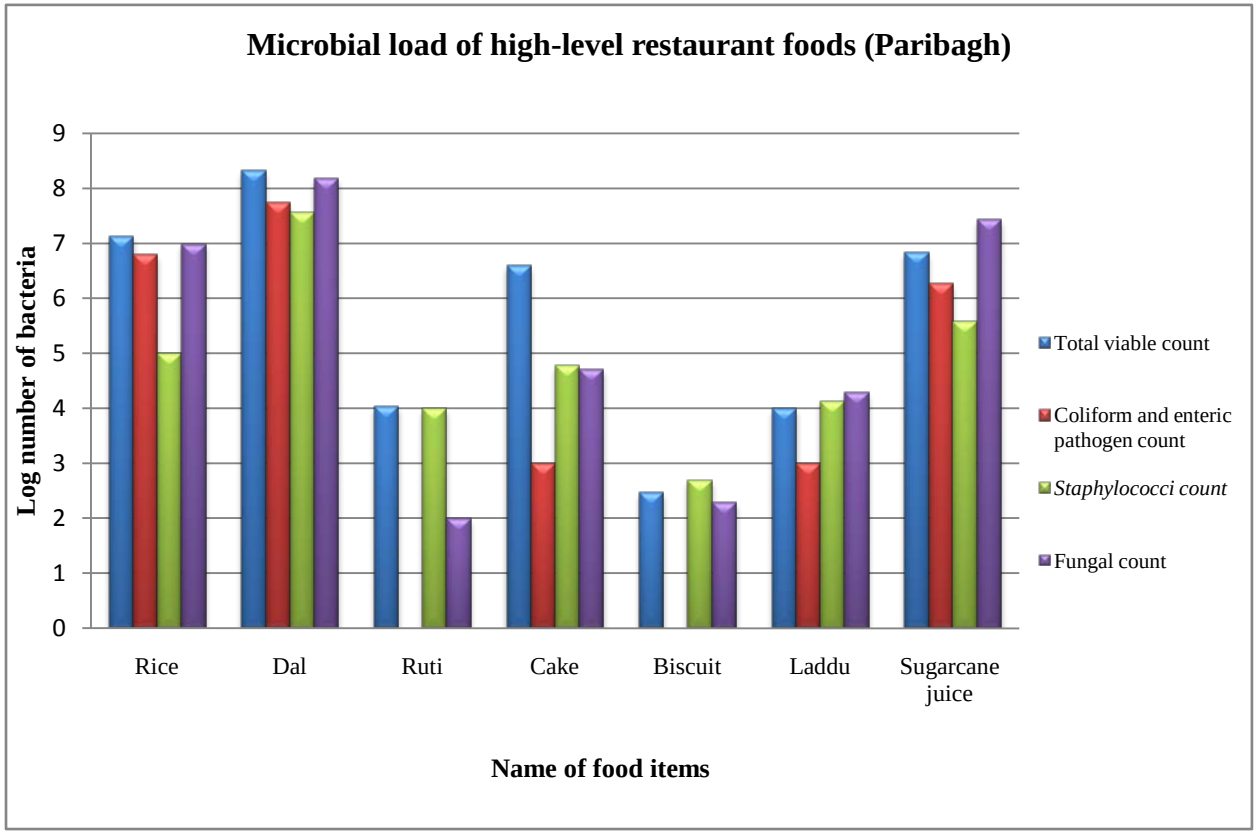

Figure-3.6: Microbial load of High-level restaurant food of Paribagh.

Rice, dal, sugarcane juice, cake and laddu have shown total four types of microbial counts. The number of food items which have shown four types of microbial count are higher than street and mid-level restaurant foods (Fig. 3.6). Only ruti and biscuit have shown three types of microbial count such as, total viable count ($1.1x10^4$, $3x10^2$), *staphylococci* count ($1x10^4$, $5x10^2$) and fungal count ($1x10^2$, $2x10^2$). In dal four types of microbial counts were higher than other food items and second highest microbial count was observed in sugarcane juice, third in rice. Lowest total viable count ($3x10^2$) and *staphylococci* count ($5x10^2$) were observed in biscuit and lowest fungal growth ($1x10^2$) was observed in ruti. Coli-form and enteric pathogen growth was not observed in ruti and biscuit.

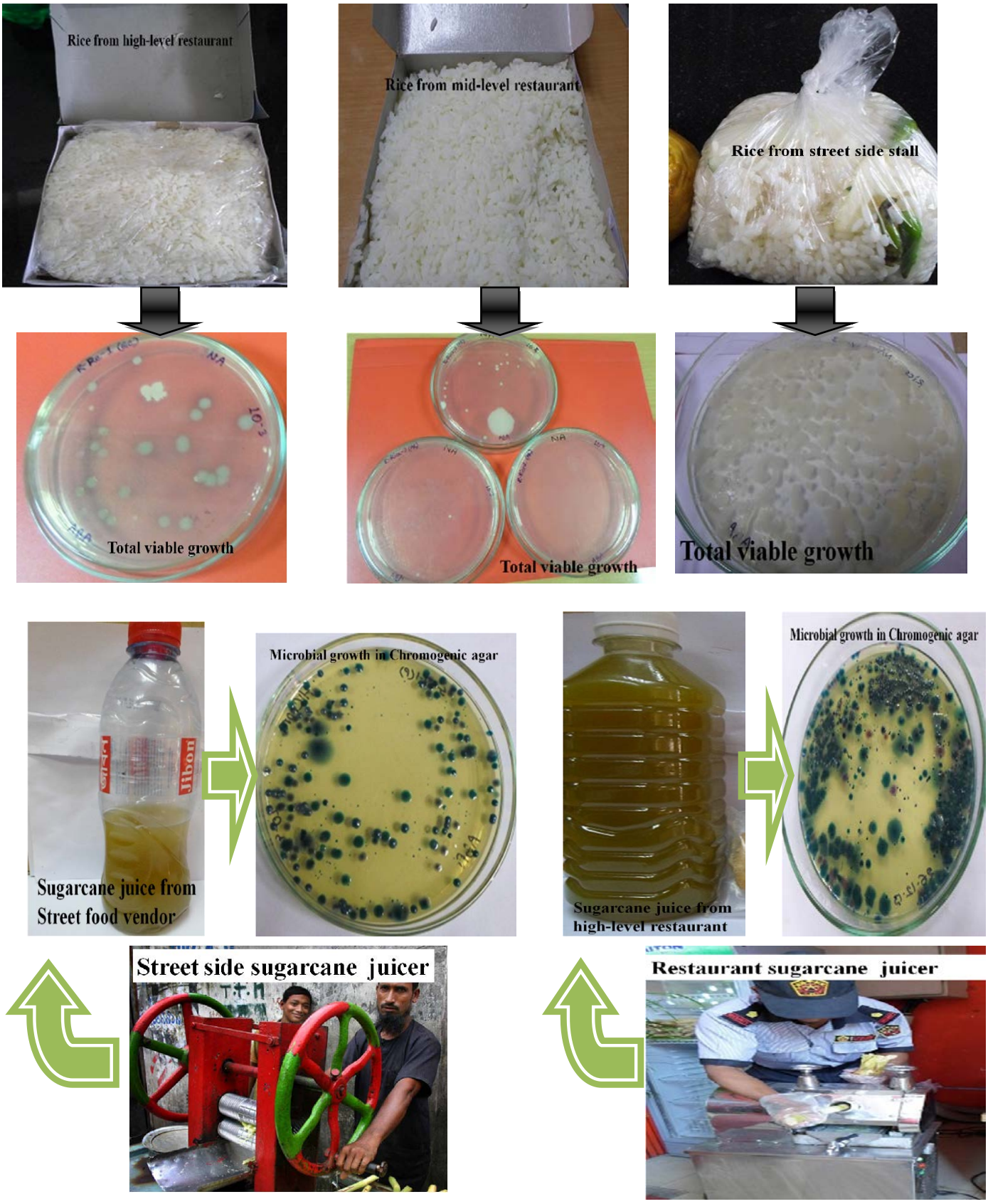

Figure 3.7: Microbial growth on different media of different food items.

3.3. Microbial load of street, mid-level and high-level restaurant's food items of Kawran bazaar area

3.3.1. Microbial load of street foods

The study was also conducted in Kawran bazar area and the strategy was the same. In this area selected food items were collected and plated for microbial load surveillance. The same pattern which was followed in Paribagh area also followed in the Kawran bazaar area. Same food item's microbial loads were analyzed for three different level of food vending location of Kawran bazaar, such as, street, mid-level restaurants and high-level restaurants. Food items were collected from Kawran Bazaar street and plated on different media to study the microbial load. Microbial status of all selected food items could not be performed due to lack of time only rice, dal, laddu and sugarcane juice were collected and plated. Below the fig-3.8 shows the microbial status of different food items collected from the street of Kawran bazaar.

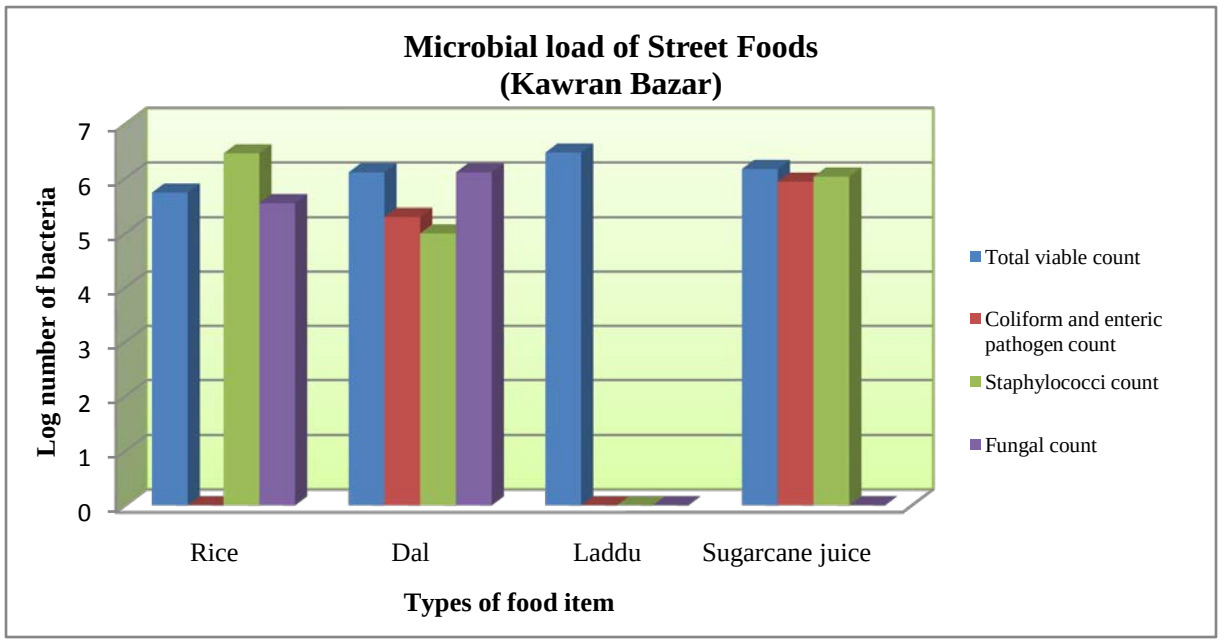

Figure-3.8: Microbial load of street food of Kawran bazaar area.

In sugarcane juice total viable count $1.5x10^6$, coli-form and enteric pathogen count $8.7x10^5$and *staphylococci* count $1.08x10^6$were observed, where total viable count is slightly higher than coli-form and enteric pathogen count and *staphylococci* count. However in rice *staphylococci* count $2.9x10^6$was higher than total viable count $5.5x10^5$ and fungal count $3.5x10^5$ but no coli-form and enteric pathogen count was detected. Laddu plating sample incubation has shown only total viable count $3x10^6$and other three types

of microbial count were not observed. The only sample which showed four types of microbial grwoth was dal. Total viable count $1.3x10^6$and fungal count $1.3x10^6$was same for dal but coliform and enteric pathogen count $2x10^5$was higher than *staphylococci* count $1x10^5$. The total viable count of laddu is higher than other food samples and the second highest was sugarcane juice.

3.3.2. Microbial load of mid-level restaurant foods

In mid-level restaurant only two food items were analyzed for their microbial growth and the food items were rice and dal. Figure 3.9 shows the growth of microbes after 24 and 48 hours.

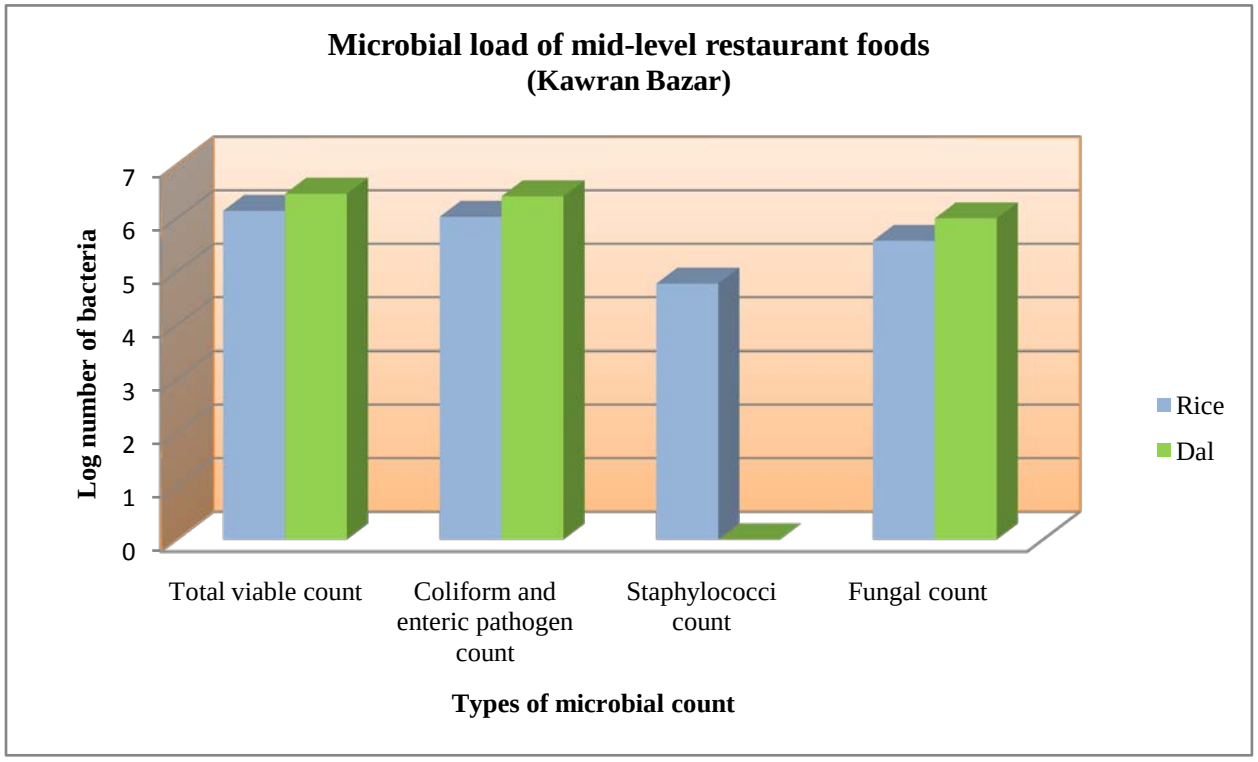

Figure-3.9: Microbial load of mid-level restaurant food of Kawran bazar area.

Total viable count $2.8x10^6$, coli form and enteric pathogen count $2.5x10^6$and fungal count $1x10^6$of dal sample was higher than the microbial load of rice sample, but no *staphylococci* count was detected in dal. However in rice sample four types of microbial growth was observed, and among them total viable count $1.3x10^6$and coli-form enteric pathogen count $1.07x10^6$ was almost same in range. Microbial count as cfu/ml was converted into logarithmic scale for proper organization of data in graphs.

3.3.3. Microbial load of high-level restaurant foods

Food samples were analyzed from high-level retaurant more than street and mid-level restaurant. Microbial loads of rice, dal, cake, biscuit and sugarcane juice were observed and the result is presented below in Fig-3.10,

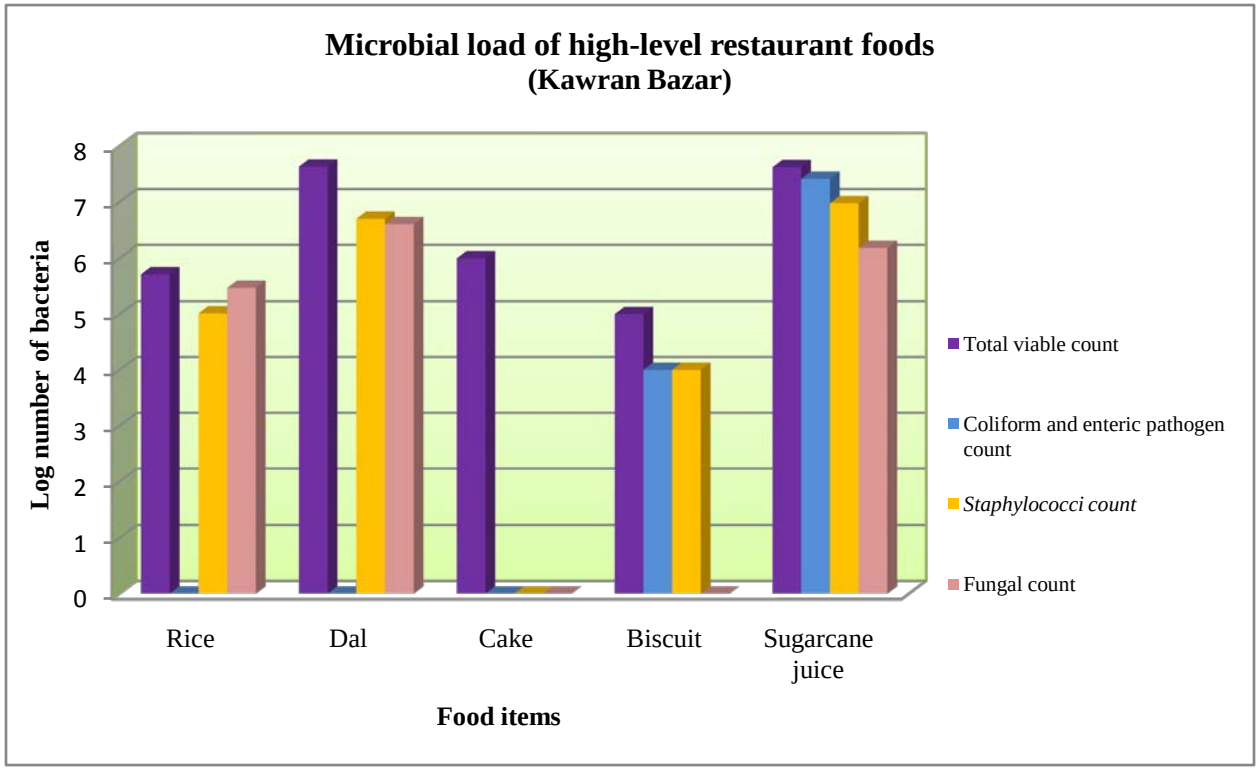

Figure-3.10: Microbial load of high-level restaurant food of kawran bazar area.

Total viable count $4.3x10^7$,*staphylococci* count$5x10^6$and fungal count $4x10^6$of dal sample was higher than other food samples of high-level restaurant except sugarcane juice sample. Only sugarcane juice sample has shown four types of microbial growth where total viable count $4.2x10^7$ was higher than coli-form and enteric pathogen count $2.6x10^7$, *staphylococci* count $9.5x10^6$ and fungal count $1.5x10^6$. Coli-form and enteric pathogen count was not observed in dal, rice and cake samples. Total viable count $1x10^6$was only observed in cake sample remaining three microbial counts were not observed. Biscuit sample has shown lowest total viable count $1x10^5$, coli-form and enteric pathogen count $1x10^4$ and *staphylococci* count $1x10^4$ compred to other food items of high-level restaurant foods. Due to shortage of time ruti and laddu samples were not analyzed for microbial growth.

3.4. Comparison of microbial load among street, mid-level and high-level restaurants

Selected food items from street, mid-level and high-level restaurants of Paribagh and Kawran bazar were analyzed for microbial growth. Different types of outcomes were observed by analyzing those foods. Total viable count, coli-form and pathogen count, *staphylococci* count and fungal count were analyzed in three categories of food shopes, such as street side stall, mid-level restaurants and high-level restaurants. These was done for only Paribagh area and it is presented in figure-3.11 below,

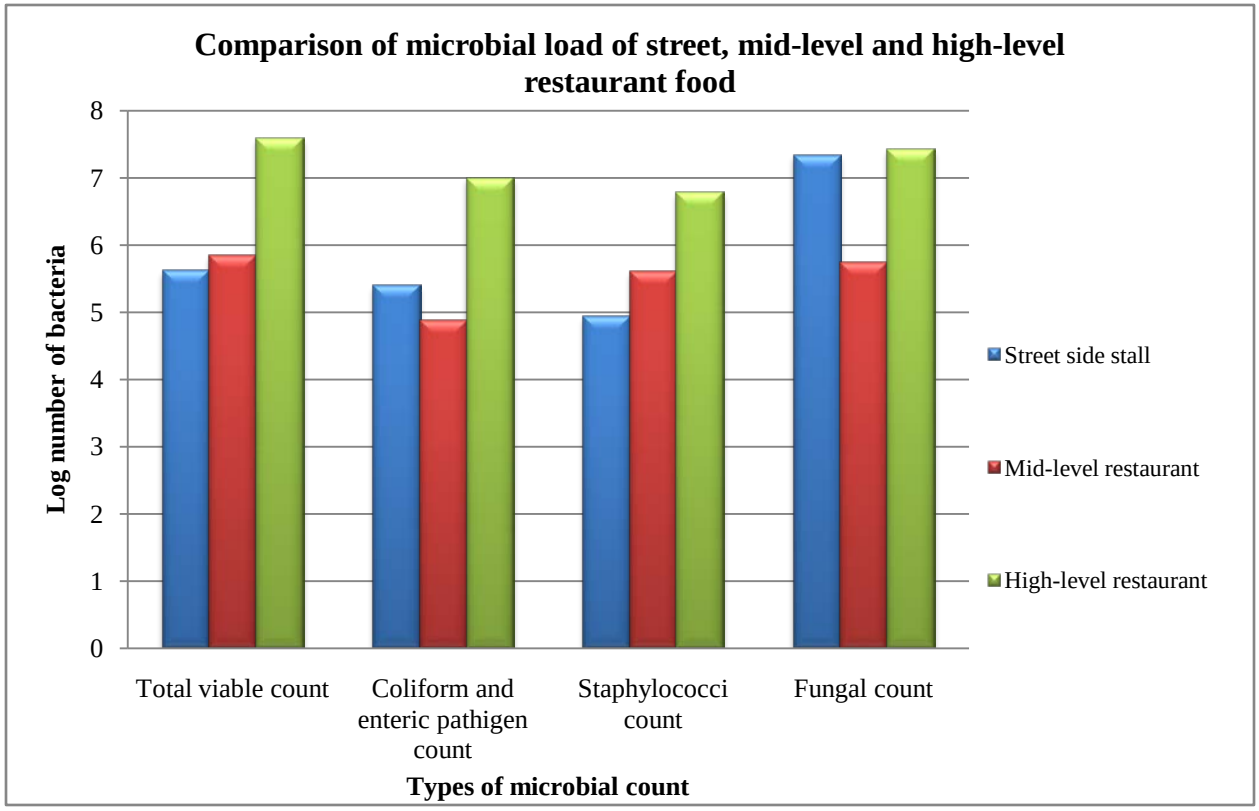

Figure-3.11: Comparison of microbial load street, mid-level and high-level restaurant's food items.

From the figure 3.11 it can be stated by observing above graph that total viable count (TVC=$3.4x10^7$>$3.9x10^6$>$7x10^5$), coli-form and enteric pathogen count (CEC=$1.2x10^7$>$5.8x10^6$>$7.5x10^4$),and fungal count (FC=$2.7x10^7$>$2.6x10^7$>$5.6x10^5$) of high-level restaurant foods were higher than street side stall and mid-level restaurant foods except *staphylococci* count (SC=$5.3x10^6$>$4.1x10^5$>$7.8x10^4$), which was lower than mid and high-level restaurant foods. Fungal count was almost same in street and high-level restaurant foods and higher than mid-level restaurant foods.

3.5. Microbial load of raw and different categories of cooked food

Microbial load of raw food and cooked food was different; as the cooking temperature kills all mesophilic microorganisms and the raw food is not cooked and consumed directly. Sometimes cooked foods also further processed by adding some raw food items, spices, oils etc to prepare specific food items and also enhance the flavor.

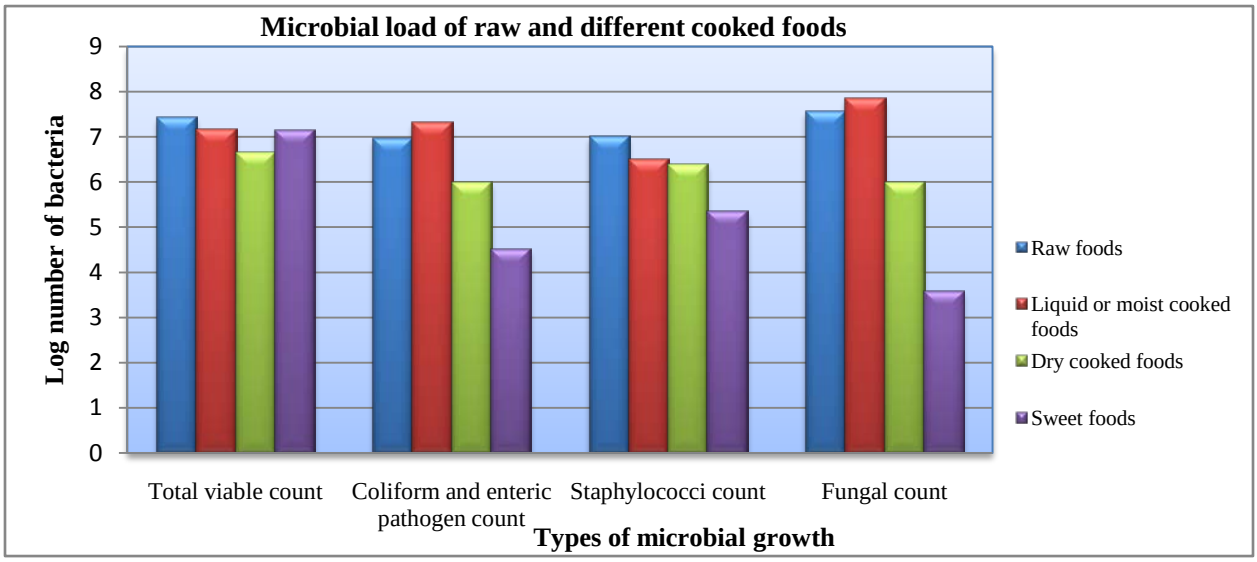

Figure-3.12: Microbial load of raw and different cooked and processed foods.

It is visible from the above figure 3.12 the total viable count (TVC) $2.7x10^7$ and *staphylococci* count $1.0x10^7$ of raw foods were higher than cooked foods. However, in case of coli-form and enteric pathogen count (CEC) $2.1x10^7$ and fungal count (FC) $7.2x10^7$, the scenario was different; these counts were higher for liquid or moist cooked foods rather than raw foods (CEC= $9.4x10^6$, FC=$3.7x10^7$). Dry cooked foods have shown low total viable count $4.6x10^6$, coli-form and enteric pathogen count $1x10^6$, *staphylococci* count $2.5x10^6$and fungal count $1.02x10^6$but moderately higher than sweet foods except total viable count $1.4x10^7$. The table below shows the categories of food items,

Table-3.4: Categories Food Items

Categories of foods	Sub-categories of foods	Name of food items
Raw food	Raw food	• Sugarcane juice
Cooked food	Liquid or moist cooked food	• Dal • Rice
	Dry cooked food	• Cake • Biscuit • Ruti
	Sweet food	• Laddu

3.5.1. Microbial load of raw and cooked foods

In previous section whole food items for experiments were categorized into four divisions, in this section microbial loads are presented for main two categories of food items (Fig-3.13).

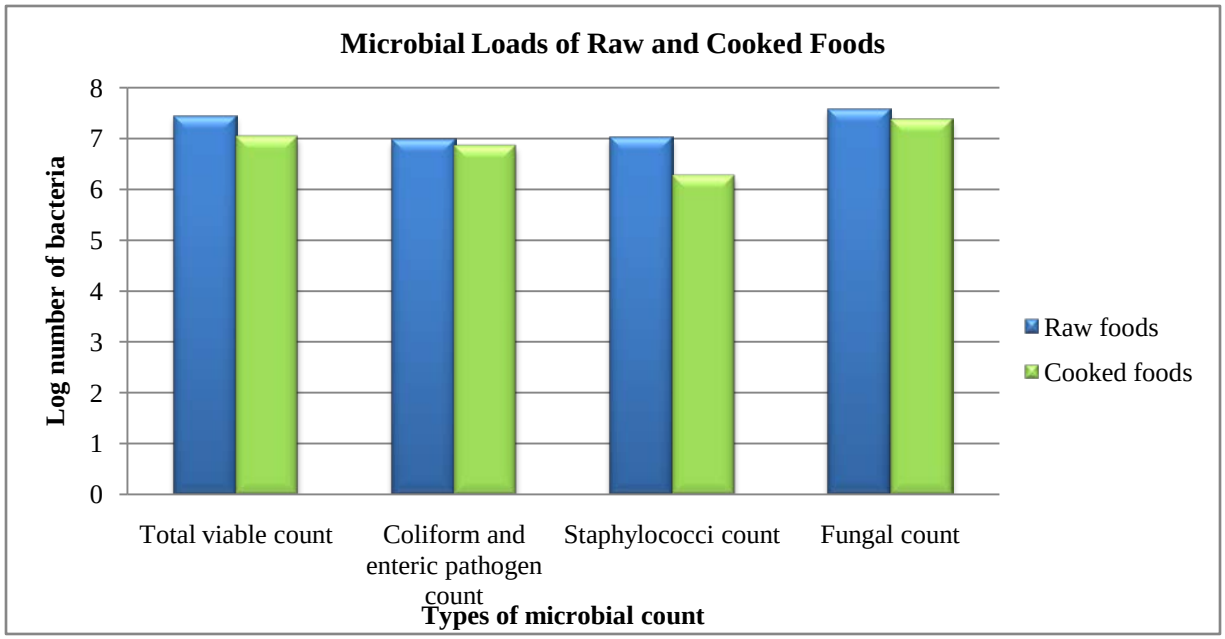

Figure-3.13: Microbial load of raw and cooked foods.

It can be stated from above figure-3.13 that microbial loads of raw foods are higher than all types of cooked foods, coli-form and enteric pathogen count (CEC= $9.4x10^7>7.3x10^7$) and fungal count (FC=$3.7x10^7>2.4x10^7$) were slightly higher than the cooked food items; total viable count (TVC=$2.7x10^7>1.1x10^7$) and *staphylococci* count (SC=$1.04x10^7>1.9x10^6$) were moderately higher than cooked foods.

3.6. Relation between water activity of food and microbial growth

The water activity food can be defined as the unbound water of food molecules which assist the growth of bacteria, fungus and mold. Moisture of food is not same as water activity; because some moist food has same moisture content but water activity is different.

In completely undisturbed surrounding air media the ratio between vapor pressure of food itself and the vapor pressure of distilled water is designated as water activity (a_w). Moisture condition of a product is Equilibrium Relative Humidity (ERH) which is expressed as percentage and the water activity expressed in decimal.

The water activity of a food is above 0.95 allow the growth of bacteria fungi and due to high moisture content.

Water activity predicts the growth of microorganisms. Refrigeration of food is not only the source of preservation, water activity (a_w) and pH can determine the growth of bacteria, fungi and mold. Water activity has relationship in microbial growth in food.

Table-3.5: Water Activity and Total Count of Selected Food items

Food item	Water activity	Total viable count(all foods of Paribagh)
Rice	0.591	6.681241
Dal	0.906	7.863323
Ruti	0.8	3.612784
Cake	0.85	6.342423
Biscuit	0.63	4.113943
Laddu	0.87	5.662758
Sugarcane juice	0.97	7.176091

IBM SPSS 20 (Software Package for Statistical Analysis) version was used for analysis of data. This software was used to determine the correlation and regression between water activity of food and total viable count of food samples. Water activity is considered as independent variable and total viable count as dependent variable. In next section SPSS Pearson correlation result is interpreted,

3.6.1. Interpretation of the linear coefficient(r)

Correlations

		Total viable count of foods (log form)	Water activity of food items
Total viable count of foods (log form)	Pearson Correlation	1	.429
	Sig. (1-tailed)		.169
	N	7	7
Water activity of food items	Pearson Correlation	.429	1
	Sig. (1-tailed)	.169	
	N	7	7

Figure-3.14: Correlation between water activity of food samples and total viable count of food samples.

The correlation coefficient is 0.429 (above Fig.3.14). The value of r suggests a moderate positive linear correlation since the value is positive. The data point should be closely clustered around

the positively sloping regression line. Therefore, it can be seen that a moderate positive linear relationship between water activity and total count of food samples.

A regression analysis was also performed to determine the effect of change of independent variables on dependent variables.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.429[a]	.184	.020	1.552578416	.184	1.125	1	5	.337

a. Predictors: (Constant), Water activity of food items
b. Dependent Variable: Total viable count of foods (log form)

Figure-3.15: Model summary.

The coefficient of determination is 0.184; therefore, about 18.4% of the variation in the total count is explained by the water activity. The regression equation appears to be moderately useful for making predictions since the value of r^2 is smaller than 1.

3.6.2. Distribution of residuals

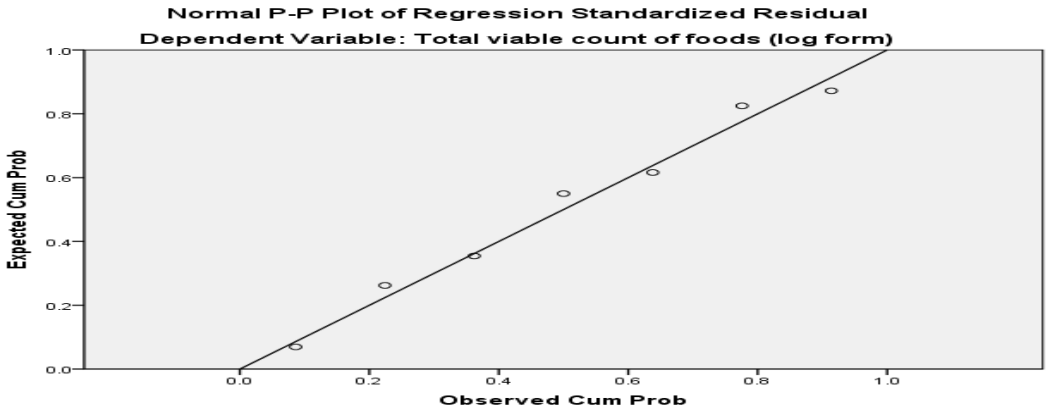

Figure-3.16: Normal probability plot of standardized residuals.

The residual plot (Fig-3.16) shows a random scatter of the points (independence) with a constant spread (constant variance). The standardized residual plot shows a random scatter of the points (independence) with a constant spread (constant variance) with a no values beyond the ±1 standard deviation reference lines (no outliers). The normal probability plot of the residuals shows the points close to a diagonal line; therefore; the residuals appear to be approximately normally distributed.

The normal distribution of residuals is also observed in histogram (Fig.-3.17) below, where residuals roughly follow the curve.

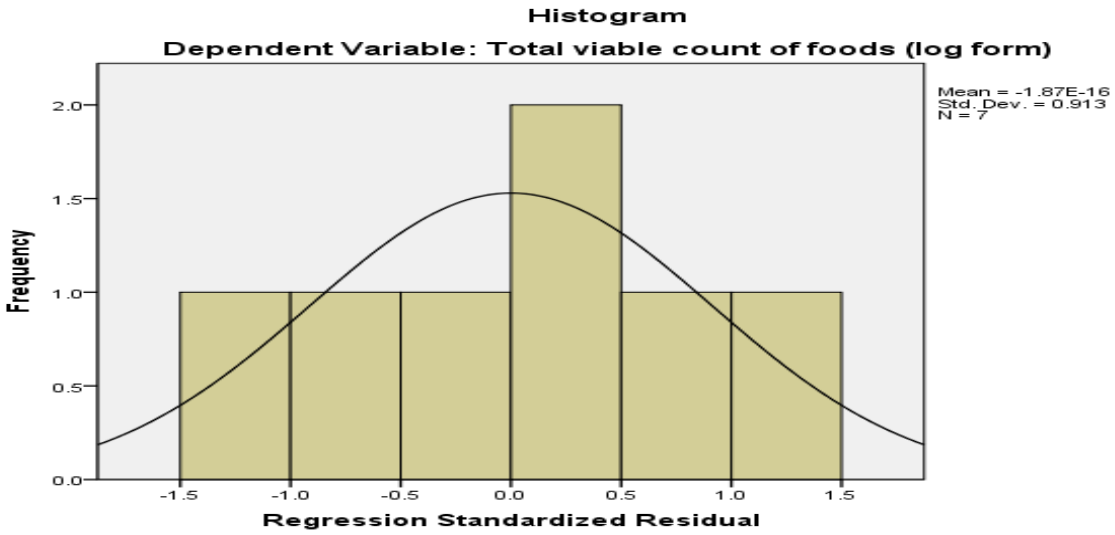

Figure-3.17: Histogram of residuals.

3.6.3. Whether water activity is useful predictor of total count of the food samples or not...

Hypotheses

H_0:β=0 (Water activity is not a useful predictor of total viable count of food.)

H_0:β≠0 (Water activity is a useful predictor of total viable count of food.)

Significance level

α= 0.05

Critical value(s) and Rejection Region(s)

Null hypothesis will be rejected if p-value ≤0.05

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.109	3.643		.579	.588
	Water activity of food items	4.752	4.480	.429	1.060	.337

a. Dependent Variable: Total viable count of foods (log form)

Figure-3.18: Coefficients of water activity.

Since p-value=0.337 ≥0.05, we shall accept the null hypothesis.

At α=0.05 level of significance, the data provided above (Fig.-3.18) proves that the slope of population regression line is not zero and water activity is not a useful predictor of total count of food items.

The main aim of this study is to analysis the microbial growth of same food samples in street, mid-level and high-level restaurants. The data size of this study is large, for this reason some data were statistically analyzed to get an overall concept about the microbial load of food samples in different collection places. Total viable count of food samples of Paribagh and Kawran Bazar's high-level restaurants were compared by performing T-test, to determine whether there is any statistical significant difference between the total counts of two areas.

3.7. Mean comparison of total count of high level restaurant foods of Paribagh and Kawran bazaar

Group Statistics

	Name of the food collection place	N	Mean	Std. Deviation	Std. Error Mean
Total viable count of food samples(High level restaurant)	Paribagh	6	5.901495655	2.200113431	.8981925471
	Kawran Bazar	6	6.408307058	1.059355306	.4324799929

Figure-3.19: Group statistics box.

In this group statistics box (Fig.-3.19) the mean for group 1(Paribagh) is 5.90 and the mean of group 2(Kawran Bazar) is 6.40. The standard deviation of group1 and 2 is 2.20 and 1.05 respectively. The participants of each group (N) are 6.

3.7.1. Levene's test for equality of variances

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Total viable count of food samples(High level restaurant)	Equal variances assumed	3.944	.075	-.508	10	.622	-.5068114030	.9968895605	-2.728019763	1.714396957
	Equal variances not assumed			-.508	7.200	.626	-.5068114030	.9968895605	-2.850860933	1.837238127

Figure-3.20: Independent samples test box.

In this box above (Fig.-3.20) there is a column named Leven's Test for Equality (red square) of Variances and this test determines two groups (1 and 2) have about same or different amounts of variability between scores. The small columns labeled F and Sig. and the value of Sig. column (red circled) is 0.075 which is greater than 0.05 this means the variability of two groups are almost same.

There is a large column labeled t-test for equality of means (green square), where it can be known that the means of two groups are statistically same or different (significantly different). The Sig. (2 tailed) value (green circled) is 0.622 which is greater than 0.05; so it can be concluded that there is no significant difference between two group's means. The slight differences in condition means are due to chance and not likely due to group total count manipulation.

Mean of total viable count of street, mid-level and high-level restaurant foods were compared by ANOVA test to evaluate whether there was any statistical difference among means.

3.8. Analysis of total count variation among street, mid-level and high-level restaurant foods

Street food, mid-level and high-level restaurant foods were considered as three groups as independent variables and total viable count of their food items were dependent variables; the output of ANOVA test is interpreted below,

ANOVA test Output:

Descriptives

Total Viable Count of different level restaurant foods

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Street Food	7	5.227427180	1.447446738	.5470834439	3.888762217	6.566092142	2.8808136	7.3944517
Mid-level Restaurant	6	4.844662836	1.662467115	.6786993580	3.100010594	6.589315077	2.6989700	6.3222193
High Level Restaurant	7	5.636328129	2.127427078	.8040918544	3.668786241	7.603870017	2.4771213	8.3384565
Total	20	5.255713209	1.710320406	.3824392691	4.455258619	6.056167799	2.4771213	8.3384565

Figure-3.21: Descriptive analysis box of ANOVA test.

Sample size (N) of (food sample) was not equal. Mean total viable count of street, mid-level and high-level restaurants are 5.22, 4.84 and 5.63 respectively and also standard deviations (Std. Deviation) are 1.44, 1.66 and 2.12 respectively. This test was performed at significance level of 0.05.

Hypotheses

H_0:β=0 (there is no significant variance amongthree group's total viable count of food.)

H_0:β≠0 (there is significant variance amongthree group's total viable count of food.)

Significance level

α= 0.05

Critical value(s) and Rejection Region(s)

Null hypothesis will be rejected if p-value ≤0.05

Test of Homogeneity of Variances

Total Viable Count of different level restaurant foods

Levene Statistic	df1	df2	Sig.
1.599	2	17	.231

Figure-3.22: Test of homogeneity of variances.

The significance value for homogeneity of variances is greater than 0.05 (Fig. 3.22), so the variances of the groups are not statistically significant. Since this is an assumption remaining results are described carefully in next section.

ANOVA

Total Viable Count of different level restaurant foods

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.033	2	1.017	.323	.728
Within Groups	53.545	17	3.150		
Total	55.579	19			

Figure-3.23: ANOVA result.

This box in Fig. 4.23 contains main ANOVA result. The significance value comparing the groups (level of restaurants) is greater than 0.05, which is 0.728, so the null hypothesis accepted; there is no significant variance among total viable count of food of three groups. However, this result does not tell us variances among each group specifically, so post hoc result will give this specification. Although statistically there are no significant differences among groups total count, this post hoc result will clarify this result statistically.

Multiple Comparisons

Dependent Variable:Total Viable Count of different level restaurant foods

	(I) Level of restaurants	(J) Level of restaurants	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Gabriel	Street Food	Mid-level Restaurant	.3827643437	.9873772986	.972	-2.215461767	2.980990454
		High Level Restaurant	-.4089009499	.9486414236	.962	-2.907047020	2.089245120
	Mid-level Restaurant	Street Food	-.3827643437	.9873772986	.972	-2.980990454	2.215461767
		High Level Restaurant	-.7916652936	.9873772986	.808	-3.389891404	1.806560817
	High Level Restaurant	Street Food	.4089009499	.9486414236	.962	-2.089245120	2.907047020
		Mid-level Restaurant	.7916652936	.9873772986	.808	-1.806560817	3.389891404

Figure-3.24: Multiple comparisons among different level restaurant's total count of foods.

The Gabriel post-hoc test does not rely on homogeneity of variances as the sample size are not equal in this test so the standard Turkey test was ignored as it relies on homogeneity of variances. In this comparison list (Fig. 3.24) significance level of each comparison is greater than 0.05, this means there are no statistically significant variance among total viable counts of three group's food items. Street food compared with mid-level where mean difference is 0.382 but significance level is 0.972 which is greater than 0.05; so, statistically there is no variance of total count of street and mid-level foods. This is also observed in remaining comparisons.

Figure-3.25: Mean total count in plot presentation.

Total viable count mean difference is observed in the above plot presentation but according to the SPSS ANOVA test this difference is not satisfy statistically, so there are no statistically significant difference among total viable count of foods of three different groups.

3.9. Biochemical test result of isolates:

Microorganisms are isolated from culture media after spreading and streaking according to their growth pattern, morphology, appearance and resemblance morphology to suspected microorganisms. The microorganisms are then subcultured and some specific biochemical tests were performed to identify these microorganisms. Biochemical test results were then put into online software which analyze the results and give four most probable names of bacteria. According to this probable microorganisms name charts were prepared. In chart two highly probable microorganism's name and their biochemical test results are put into table. All samples were not biochemically tested as the sample size was large and different types of biochemical tests have to be performed. For this reason each sample from each level of food samples collection places were selected and biochemical tests were performed. The types of biochemical tests which were performed are described precisely in materials and method chapter. Biochemical test results are given in next section.

Table-3.6: Results of standard biochemical test for identification of unknown microorganisms (high-level restaurant).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
H-Rc-3																
Hi-1	-coccibacilli	+	AAG	-	+	-	AG	AG	±	+	+	-	-	+	±	*Serratia liquifaciens 92% (32%), Cedecea species 5 89% (32%)*
Hi-3	-cocci	+	AAG-	-	+	-	AG	A	-	+	-	-	-	-	-	*Tatumella ptyseos 79% (32%), Conobacter sakazakii 70%*
Hi-4	-bacilli	-	BAG-	-	-	+	AG	A	-	+	-	-	-	-	-	*Pseudomonas fluorescens biovar 1 83% (34%)*
Ms-1	-cocci	+	AAG-	-	+	-	AG	A	A	-	+	-	+	+	-	*Pseudomonas oryzihabitans 75% (28%)*
Ms-2	+coccobacilli	+	AAG-	-	+	-	AG	A	-	-	-	-	-	±	+	*Staphylococcus aureus subsp. aureus 81% (22%), Staphylococcus carnosus subtilis 81% (22%)*
TCBS	-coccobacilli	+	AAG+	-	+	-	AG	AG	-	+	+	-	-	+	+	*Vibrio furnissi 83% (28%)*
H-DL-3																
Hi-1	-cocci	+	AAG-	+	+	-	AG	AG	-	-	-	-	+	+	+	*Enterobacter pyrinus 78% (32%), Proteus penneri 73% (32%)*
Hi-2	-coccobacilli	+	BBG-	+	+	-	AG	AG	-	-	±	-	+	+	-	*Proteus penneri 85% (32%), Proteus vulgaris 85% (32%)*
EM-1	-coccobacilli	+	BBG-	+	+	-	AG	AG	-	-	+	-	+	+	-	*Proteus penneri*
EM-2	-coccobacilli	+	AAG+	+	+	-	AG	AG	-	-	+	-	+	+	+	*Proteus penneri*
NA	-coccobacilli	+	AAG-	+	+	-	AG	A	-	-	+	-	+	+	-	*Proteus penneri 91% (30%), Proteus vulgaris 91% (30%)*
TCBS	-cocci	+	AAG-	+	+	-	AG	AG	-	-	+	-	+	+	+	*Proteus penneri*
MSA	-cocci	+	AAG-	+	+	-	AG	AG	-	+	+	-	+	+	+	*Cosenzea (Proteus) myxofaciens 85% (30%)*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, Hi=HiCrome, Rc=rice, DL=Dal, H=high level restaurant

Table-3.7: Results of standard biochemical test for identification of unknown microorganisms (high-level restaurant).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
H-RT																
Hi-1	+bacilli	+	AAG-	-	+	+	AG	A-	-	-	+	-	+	+	+	*Bacillus psychrosaccharolyticus 84% (26%), Bacillus subtilis 82% (26%)*
Hi-2	-bacilli	-	AAG-	-	+	+	A-	A-	-	+	+	-	-	-	-	*Pseudomonas fluorescens biovar 1 99% (28%)*
MSA	-coccus	+	AAG-	-	+	-	A-	A-	-	-	-	-	+	-	-	*Staphylococcus gallinarum 91% (24%), Staphylococcus equorum87% (24%)*
P-lad																
MSA	+cocci	+	AAG-	-	+	-	A-	A	±	-	+	-	+	+	-	*Staphylococcus aureus subsp. aureus 91% (24%), Staphylococcus chromogens 91% (24%)*
Mac	+cocci	-	BBG-	-	+	-	AG	-	-	-	±	-	-	-	-	*Staphylococcus cohnii 81% (22%), Micrococcus bovicus 80% (22%)*
EM-1	-coccobacilli	+	AAG+	-	+	-	AG	AG	-	-	+	-	-	+	-	*Vibrio sinaloensis 83% (32%), Vibrio cincinatiensis 82% (26%)*
EM-2	-coccobacilli	-	BBG-	-	+	-	A	-	-	+	-	-	-	+	-	*Xenorhubdus beddingii 83% (32%), Xenorhubdus poinarii 83% (32%)*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, Hi=HiCrome, H=high level restaurant, RT=Ruti, P=Premium.

Table-3.8: Results of standard biochemical test for identification of unknown microorganisms (high-level restaurant).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
H-Ck-3																
Hi-1	-cocci	-	BAG-	-	+	-	A	A	-	-	-	-	-	+	-	*Pantoea stewartii subsp. stewartii 95% (28%), Arsenophonus nasoniae 85% (30%)*
Hi-2	+cocci	+	BBG-	-	+	-	A	A	-	-	+	-	-	+	±	*Staphylococcus aureus subsp. aureus 81% (22%), Staphylococcus carnasus subsp. utilis 81% (22%)*
Hi-3	+cocci	-	AAG-	-	-	+	A	A	-	-	-	-	+	-	-	*Staphylococcus equorum subsp. equorum 84% (20%), Staphylococcus gallinarum 84% (20%)*
EM-1	+cocci	-	AAG-	-	+	-	A	A	-	-	-	-	-	+	-	*Pantoea stewartii subsp. stewartii 95% (28%), Arsenophonus nasoniae 85% (30%)*
EM-2	-cocci	+	AAG-	-	+	-	A	A	-	-	-	-	-	-	-	*Tatumella ptyseos 92% (30%), Arsenophonus nasoniae 85% (30%)*
TCBS	-cocci	-	AAG-	-	+	-	A	A	-	-	-	-	-	+	+	*Pantoea stewartii subsp. stewartii 88% (28%)*
MSA	+cocci	-		-	+	-	A	A	-	-	-	-	-	-	-	*Staphylococcus arlettae 91% (30%), Macrococcus lamae 87% (24%)*
H-Bis-3																
MSA	-bacilli	-	AAG-	-	+	+	A	A	-	-	-	-	-	-	-	*Sphingomonus paucimobilis 99% (20%), Moraxella pluranimalium 89% (28%)*
NA	-cocci	-	NC	-	+	-	A	A	-	-	-	-	-	-	-	*Tatumella ptyseos 86% (32%),*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, Hi=HiCrome, H=high level restaurant, Ck=Cake, Bis= Biscuit.

Table-3.9: Results of standard biochemical test for identification of unknown microorganisms (high-level restaurant).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
HR-SJ-2																
NA-1	-coccobacilli	+	BAG	-	+	-	AG	A-	A	-	±	-	-	+	+	*Kluyvera intermedia 85% (30%), Cronobacter sakazakii 84% (30%)*
NA-2	+bacilli	+	BBG-	-	+	-	AG	AG	-	-	-	-	-	-	-	*Bacillus neizhounsis 91% (22%), Bacillus amylolyticus 91% (22%)*
EM-1	-coccobacilli	+	BAG+	-	+	-	AG	AG	-	-	+	-	-	+	+	*Tatumella terrea 86% (32%), Pantoea dispersa 85% (32%)*
EM-2	+coccobacilli	+	AAG-	-	+	-	AG	AG	-	-	+	-	-	+	+	*Bacillus acidiproducens 91% (22%), Bacillus siamensis 91% (22%)*
Mac	-coccobacilli	+	AAG-	-	+	-	AG	AG	-	-	+	-	-	+	+	*Serratia liquifaciens 85% (32%), Cedecea species 5 83% (32%)*
XLD	-coccobacilli	+	BAG+	-	+	-	AG	AG	-	-	+	-	-	+	-	*Escherichia vulnaris 89% (34%), Escherichia hermanni 87% (34%)*
TCBS	-coccobacilli	+	AAG+	-	+	-	A-	A-		-	+	-	-	+	+	*Serratia liquifaciens 91% (30%), Cedecea species 5 89% (30%)*
MSA	+cocci	+	ABG-	-	+	-	A-	A-	AG	-	-	-	-	-	-	*Staphylococcus chromogens 90% (22%), Staphylococcus arlettae 90% (22%)*
Hi-3	-bacilli	+	ABG-	-			A	AG	-		-	-	-	-	-	*Tatumella ptyseos 92% (32%), Klebsiella pneumoniae subsp. rhinoscleromatis 85% (32%)*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, **TCBS**=Thiosulfate-citrate-bile salts-sucrose agar, **XLD**=Xylose lysine deoxycholate, **Hi**=HiCrome, **H**= high-level restaurant, **SJ**= Sugarcane Juice.

Table-3.10: Results of standard biochemical test for identification of unknown microorganisms (mid-level restaurant).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
M-Rc-3																
N-1	-cocci	+	NC	-	+	+	A	-	-	-	-	-	-	+	+	*Bacillus acidiceler* 91% (24%), *Bacillus neizhouensis* 84% (24%)
N-2	+cocci	-	NC	-	+	-	--	-	-	-	N	-	N	-	-	*Obesumbacterium proteus* 84% (30%)
TCBS	+cocci	+	AAG+	-	+	-	AG	AG	-	±	-	-	+	+	+	*Enteric group 68* 83% (32%), *Tatumella punctata* 79% (32%)
M-DL-3																
Hi	-bacilli	-	AAG-	-	-	+	A	AG	-	-	±	-	-	+	-	*Burkholderia caryophelli* 79% (28%), *Pseudomonas fluorescens biovar* 1 79% (28%)
Mc-1	-bacilli	+	BBG-	-	+	-	A	A	-	-	±	-	-	+	-	*Edwardsiella hoshinae* 82% (32%), *Klebsiella pneumonia* subsp. *rhinoscleromatis* 79% (32%)
Mc-2	-bacilli	-	BBG-	-	+	-	A	A	-	±	-	-	-	+	-	*Pantoea stewartii* subsp. *stewartii* 89% (30%), *Tatumella punctata* 79% (32%)
Mc-3	-coccobacilli	+	AAG+	-	+	-	AG	A	-	±	+	-	-	+	+	*Serratia liquifaciens* 85% (32%), *Cedecea* species 83% (32%)
TCBS	-bacilli	+	AAG+	-	+	-	AG	AG	-	±	-	-	-	+	+	*Rahnella aquatillis* 91% (30%), *Serratia plymuthica* 87% (32%)
MSA	+bacilli	+	AA G-	-	+	-	A	A	-	-	-	-	-	+	-	*Bacillus neizhouensis* 91% (24%), *Paenibacillus amylolyticus* 91% (24%)
M-RT-3																
Hi-1	-cocci	-	AAG-	-	-	-	A	A	-	-	-	-	-	+	+	*Pantoea stewartii* subsp. *stewartii* 89% (30%), *Enteric group 68* 76% (32%),
Hi-2	+bacilli	+	AAG-	-	+	-	A	A	-	-	+	-	-	+	+	*Bacillus thuringiensis* 90% (33%), *Bacillus cereus*88% (33%)

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, Hi=HiCrome, M=Mid-level Restaurant, Rc=Rice, Dl=Dal, RT=Ruti.

Table-3.11: Results of standard biochemical test for identification of unknown microorganisms (mid-level restaurant).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
M-Ck-3																
EM	-bacilli	+	BA-G-	-	+	-	-	-	-	-	-	-	-	-	-	*Obesumbacterium proteus 77% (32%), Shimwellia pseudoproeus*
TCBS	+cocci	+	AAG-	-	+	-	AG	AG	-	-	-	-	+	+	+	*Staphylococcus aureus subsp. aureus 90% (22%), Staphylococcus pseudintermedius 86% (22%)*
NA	+bacilli	+	B-AG	-	+	-	AG	AG	-	-	+	-	-	-	+	*Bacillus cereus 91% (33%), Bacillus mycoides 89% (33%)*
SDA	+bacilli	+	AAG-	-	+	-	A	A	-	-	±	-	-	-	-	*Paenibacillus amylolyticus 92% (22%) Paenibacillus chibensis 92% (26%)*
M-Bis-3																
Hi	-bacilli	+	BA-	-	-	-	A	A	-	-	+	-	-	-	-	*Xenorhubdus japonica 83% (32%), Tatumella ptyseos 79% (32%)*
NA		+	NC	-	+	-	A	A	-	-	-	-	-	-	+	
SDA	+cocci	+	AAG-	-	+	-	A	A	-	-	+	-	-	+	-	*Staphylococcus muscae 90% (26%), Staphylococcus chromogens 86% (22%)*
M-Ld-1																
NA-1	+bacilli	+	ABG-	-	+	-	AG	A	±	-	±	-	-	-	-	*Paenibacillus messiliensis 99% (22%), Bacillus nealsonii 92% (26%)*
NA-2	+cocci	+	NC	-	+	-	A	A	-	-	-	-	-	-	-	*Staphylococcus haemolyticus 80% (24%), Staphylococcus muscae 74% (24%)*
NA-3	+cocci	+	ABG-	-	+	-	A	A	-	-	-	-	-	-	-	*Staphylococcus arlettae 83% (24%), Macrococcus lamae 86% (24%)*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, Hi=HiCrome, M=Mid-level Restaurant, Ck=Cake, Bis=Biscuit, Ld=Laddu.

Table-3.12: Results of standard biochemical test for identification of unknown microorganisms (street food).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
S-Rc-1																
Hi-1	+bacilli	+	ABG-	-	-	-	A	A	AG	-	+	-	-	+	+	*Bacillus siamensis 82% (26%), Bacillus cereus 82%(26%)*
Hi-2	+bacilli	+	AAG+	-	-	-	AG	A	AG	-	-	-	-	+	+	*Bacillus siamensis 84% (24%), Paenibacillus polymyxa 83%(24%)*
Hi-3	-bacilli	+	AAG-	-	-	+	AG	A	N	-	-	-	-	+	+	*Pseudomonas fluorescence biovar 4 79% (28%), Sphingomonas paucimobilis 79% (28%)*
NA	+bacilli	+	AAG-	-	-	-	A	AG	N	-	+	-	-	+	+	*Bacillus cereus 88% (24%), Bacillus acidiceler 83% (24%)*
XLD	-coccobacilli	+	AAG+	-	+	-	AG	AG	AG	+	+	-	±	+	+	*Cosenza(Proteus)myxofaciens86%(32%), Cronobacter sakazakii 85% (32%)*
S-Dl-1																
Hi-1	-cocci	+	AAG-	-	+	-	AG	AG	N	-	+	-	-	-	-	*Edwardsiella hoshinae 82% (32%), Escherichia vulneris 81% (30%)*
Hi-2	-cocci	+	AAG-	-	+	-	AG	AG	-	-	-	-	-	-	-	
Hi-3	+cocci	+	AAG-	-	+	-	AG	A	-	-	-	-	-	-	-	*Staphylococcus muscae 90% (22%), Staphylococcus Chrmogens 86% (22%)*
EM-1	-coccobacilli	+	AAG-	-	+	±	-	-	-	-	-	-	+	+	+	*Shigella dysentriae 76% (32%), Hafnia alvei biogroup1 76% (32%)*
EM-2	-coccobacilli	+	AAG-	-	+	-	AG	-	-	-	+	-	±	+	+	*Escherichia vulneris 82% (32%), Enteric group 60 82% (32%)*
MS-1	+cocci	+	AAG-	-	+	-	AG	AG	-	-	-	-	-	+	-	*Staphylococcus saprophyticus subsp. Bovis 82% (22%), Staphylococcus capitis subsp. Capitis 81% (22%)*
MS-2	+cocci	+	AAG-	-	+	-	A	AG	-	-	-	-	-	-	-	*Staphylococcus arlettae 89% (20%), Staphylococcus muscae 89% (20%)*
XLD	-coccobacilli	+	AAG+	-	+	-	AG	AG	AG	+		-	-	+	+	*Rahnella aquatilis 91% (32%),Serratia plymuthica 87% (32%)*
TCBS	-coccobacilli	+	AAG+	-	+		AG	AG	A	+	+	-	-	+	+	*Cronobacter sakazakii 92% (32%), Serratia rubidaea 89% (32%)*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, Hi=HiCrome, S=Street, Rc=Rice, Dl=Dal.

Table-3.13: Results of standard biochemical test for identification of unknown microorganisms (street food).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
S-Lad-1																
Hi-1	-diplococci	-	AAG-	-	+	-	A	NC	A	-	-	-	+	+	-	*Staphylococcus arlettae 89%(20%), Staphylococcus cohnii subsp. Urilyticus 84% (20%)*
Hi-2	+cocci	+	NC	-	+	-	N	AG	N	-	-	-	-	-	-	*Staphylococcus muscae 89% (20%), Staphylococcus chromogenes 75% (20%)*
NA	+cocci	+	NC	-	+	-	N	N	N	-	-	-	±	-	-	*Staphylococcus equorum subsp. Linens 81% (22%), Staphylococcus saprophyticus 80% (22%)*
TCBS	-cocci	+	AAG-	-	+	-	A	A	A	-	-	-	±	+	+	*Raoultella planticola 79% (32%), Yersinia bercoveri 77% (32%)*
MSA	+cocci	+	AAG-	-	+	-	A	A	A	-	-	-	+	+	+	*Staphylococcus aureus 99% (22%), Staphylococcus chromogenes 86% (22%)*
SD-2	+bacilli	-	NC	-	+	-	N	N	N	-	-	-	-	-	-	Unknown
S-Bis-3																
EMB	-bacilli	+	AAG+	-	+	-	AG	A	A G	-	+	+	-	+	-	*Pseudomonas flourescens biovar 4 72% (31%),*
TCBS	+cocci	-	AAG -	-	+	-	A	N	N	-	-	-	-	+	+	*Staphylococcus cohnii subsp. cohnii 81% (22%)*
Hi-1	+cocci	-	NC	-	+	-	A	A	A	-	-	-	-	+	-	*Staphylococcus arlettae 90% (22%)*
Hi-2	+coccobacilli	-	AAG+	+	+	-	AG	A	A G	+	+	-	-	-	-	*Bacillus megaterium 91% (24%), Bacillus nealsonii 91% (24%)*
MSA	cocci	-	NC	-	+	-	A	N	N	-	-	-	-	-	-	*Xenorhubdus japonica 89% (32%), Obesum bacterium proteus 85% (32%)*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, **Hi**=HiCrome, **Ld**= Laddu, **Bis**=Biscuit, **S**=Street.

Table-3.14: Results of standard biochemical test for identification of unknown microorganisms (street food).

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
S-Ck-3																
Hi-1	-bacilli	+	AAG-	-	+	-	AG	A	A G	-	+	+	-	+	-	*Escherichia hermannii 92% (32%), Escherichia coli 89% (32%)*
Hi-2	-bacilli	+	AAG+	-	+	-	AG	N	A G	-	+	+	-	+	-	*Escherichia hermannii 92% (32%), Escherichia coli 89% (32%)*
Ms-2	-coccobacilli	+	AAG+	-	-	+	AG	N	A G	-	+	+	-	+	-	*Pseudomonas flourescens biovar 4 79% (28%), Pseudomonas aeroginosa 75% (28%)*
TCBS	+cocci	+	AAG-	-	+	-	A	A	N	-	-	-	+	-	-	*Staphylococcus gallinarum 90% (22%), Staphylococcus saprophyticus subsp. bovis 87% (22%)*
SDA	+bacilli	+	AAG+	-	+	-	AG	A	A G	-	+	+	-	+	-	*Escherichia hermannii 92% (32%), Escherichia coli 89% (32%)*
S-Rt-1																
NA	+bacilli (sp)	-	AAG-	-	+	-	A	N	N	+	-	-	+	-	-	*Bacillus mycoides 83% (24%), Paenibacillus apiaries 80% (24%)*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, **Hi**=HiCrome, S=Street, Ck=Cake, Rt=Ruti.

Table-3.15: Results of standard biochemical test for identification of unknown microorganisms (street food)

Sample name	Gram stain	Nitrate	TSI $S_{lant}B_{ut}G_{as}$	H_2S production	Catalase	Oxidase	Dextrose	Sucrose	Lactose	Citrate	Motility	Indole	Urease	Methyl red	Voges-Proskauer	Suspected organism
S-SJ-2																
Hi-1	-coccobacilli	+	AAG+	-	+	-	AG	AG	A G	-	-	-	-	+	+	*Raoultella terrigena 85% (32%), Rahnella aquatillis 85% (32%)*
Hi-2	-bacilli	+	AAG+	-	+	-	AG	AG	A G	-	±	-	-	+	+	*Kueyvera intermedia 86% (32%), Cronobacter sakazaii85% (32%)*
Hi-3	+bacilli	+	BBG-	-	+	+	N	N	N	-	±	-	-	-	-	*Limenorella richardii 76% (28%), Obesumbacterium proteus 76% (28%)*
EMB	-coccobacilli	+	AAG-	-	+	-	AG	AG	A G	-	-	-	-	+	+	*Raoultella terrigena 85% (32%),*
TCBS	+coccobacilli	+	AAG+	-	+	-	AG	AG	A G	-	-	-	+	+	+	*Photobacter phosphoreum 67% (28%), Aeromonas salmonicida subsp. masoucida 65% (28%)*
MSA	+coccobacilli	+	AAG-	-	+	-	AG	AG	A G	-	-	-	+	+	+	*Staphylococcus aureus subsp. aureus 99% (24%), Staphylococcus simulans 83% (24%)*
XLD	-cocci	+	AAG+	+	+	-	AG	AG	A G	-	+	-	±	+	+	*Proteus mirabilis 73% (32%), Citrobacter ferundii 73% (32%)*

'A'= acidic, 'B'=basic, 'G'=gas, '+'= positive, '-'=negative, NC=no change, **NA**=Nutrient Agar, **Mac**=Macconkey Agar, **EMB**=Eosine Methylene Blue Agar, **MSA**=Manitol Salt Agar, **SDA**=Saburaoud Dextrose Agar, TCBS=Thiosulfate-citrate-bile salts-sucrose agar, XLD=Xylose lysine deoxycholate, **Hi**=HiCrome, S=Street, SJ=Sugarcane Juice.

3.10. Presumptive pathogens from food samples

In previous section it was mentioned that all names of bacteria are the most probable result of ABIS online software after input the biochemical results. There are two percentages beside each name of bacteria one is for the probability percentage and another one enclosed within parentheses is for the accuracy of the matching of biochemical results with the encyclopedia of the software. In this section the probability of the results are analyzed briefly. All microorganisms are not pathogenic so, pathogenic and non-pathogenic microorganisms are counted and percentages of them were also calculated. Pathogens are categorized into three sections such as, invasive pathogen, opportunistic pathogen and rare pathogen. Some pie charts and tables are made from these results and all of them are sequentially described below.

Table-3.16: Types of presumptive pathogens in street, mid-level and high-level restaurant foods.

Type of pathogen	**Name of Total Pathogens**	**Street**	**Mid-level Restaurant**	**High-level Restaurant**
Invasive	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus*
	Bacillus cereus	*Bacillus cereus*	*Bacillus cereus*	
	Shigella dysentriae	*Shigella dysentriae*		
	Proteus mirabilis	*Proteus mirabilis*		*Proteus mirabilis*
Opportunistic	*Cronobacter sakazakii*	*Cronobacter sakazakii*		*Cronobacter sakazakii*
	Pseudomonas fluorescens biovar 1	*Pseudomonas fluorescens* biovar 1		*Pseudomonas fluorescens* biovar 1
	Serratia liqfaciens		*Serratia liqfaciens*	
	Sphingomonas paucimobilis			*Sphingomonas paucimobilis*
	Staphylococcus cohnii subsp. *cohnii*	*Staphylococcus cohnii* subsp. *cohnii*		
	Staphylococcus haemolyticus	*Staphylococcus haemolyticus*	*Staphylococcus haemolyticus*	
	Tatumella ptyseos		*Tatumella ptyseos*	*Tatumella ptyseos*
	Proteus penneri			*Proteus penneri*
	Bacillus subtilis			*Bacillus subtilis*
	Escherichia hermannii	*Escherichia hermannii*		
Rare	*Staphylococcus gallinarum*			*Staphylococcus gallinarum*
	Rahnella aquatilis	*Rahnella aquatilis*	*Rahnella aquatilis*	

Invasive bacterial pathogens are those that can invade the parts of the body where normally other bacteria cannot enter such as blood stream, soft tissues like muscle and fat, brain tissues. **Opportunistic bacterial pathogens** are not capable infect healthy people; it can infect through any kind of wound, operation parts of the human body and also infect diseased people. **Rare pathogens** are those which can infect immune compromised people but normally not capable of infect healthy person.

In this study three categories of pathogens were identified and few invasive and opportunistic bacterial pathogens were then further confirmed by specific biochemical tests. These three categories of bacteria were analyzed graphically (Fig.-3.26), to find out the number of pathogenic and non-pathogenic bacteria and their percentages. Total 95 isolates were biochemically identified among them 54 isolates are non-pathogen and 41 isolates are pathogenic bacteria.

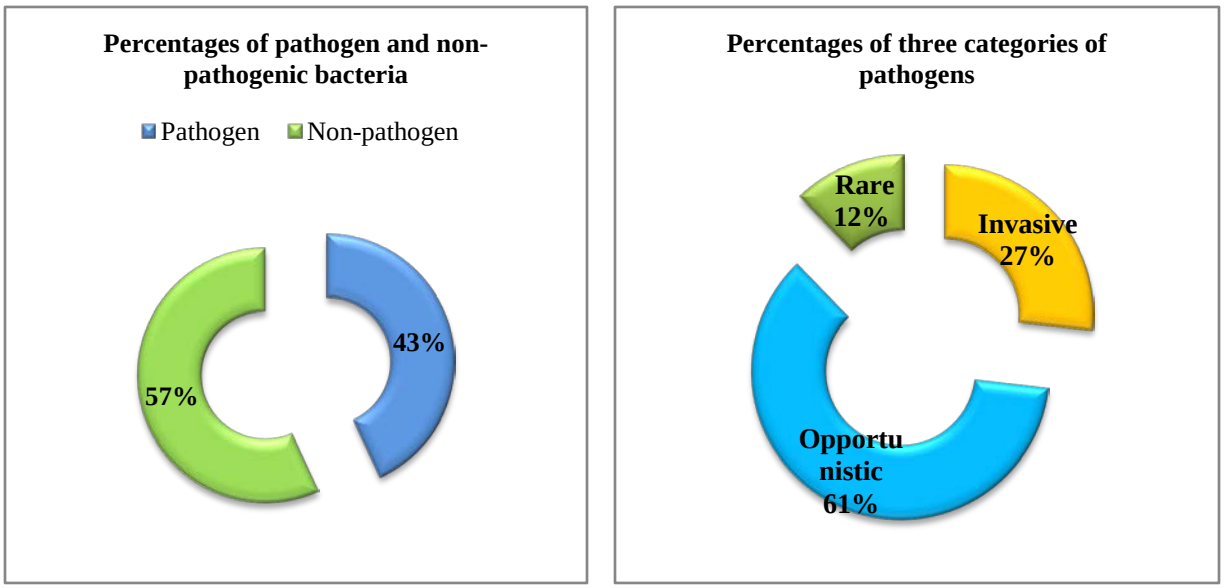

Figure-3.26: Pathogenic and non-pathogenic bacteria (left side) and percentages of three categories of pathogens (right side).

Around 43% pathogens are identified from food samples which is less than 57%, the percentage of non-pathogenic microorganisms. As mentioned above there are three categories of pathogens which are identified among them percentage of opportunistic pathogen 61% is more than double of invasive pathogen percentage 27% and remaining 12% is rare pathogens.

Presumptive pathogenic bacteria from food sample of street, mid and high level restaurants are different and their percentages are graphically presented below in Fig. 3.27,

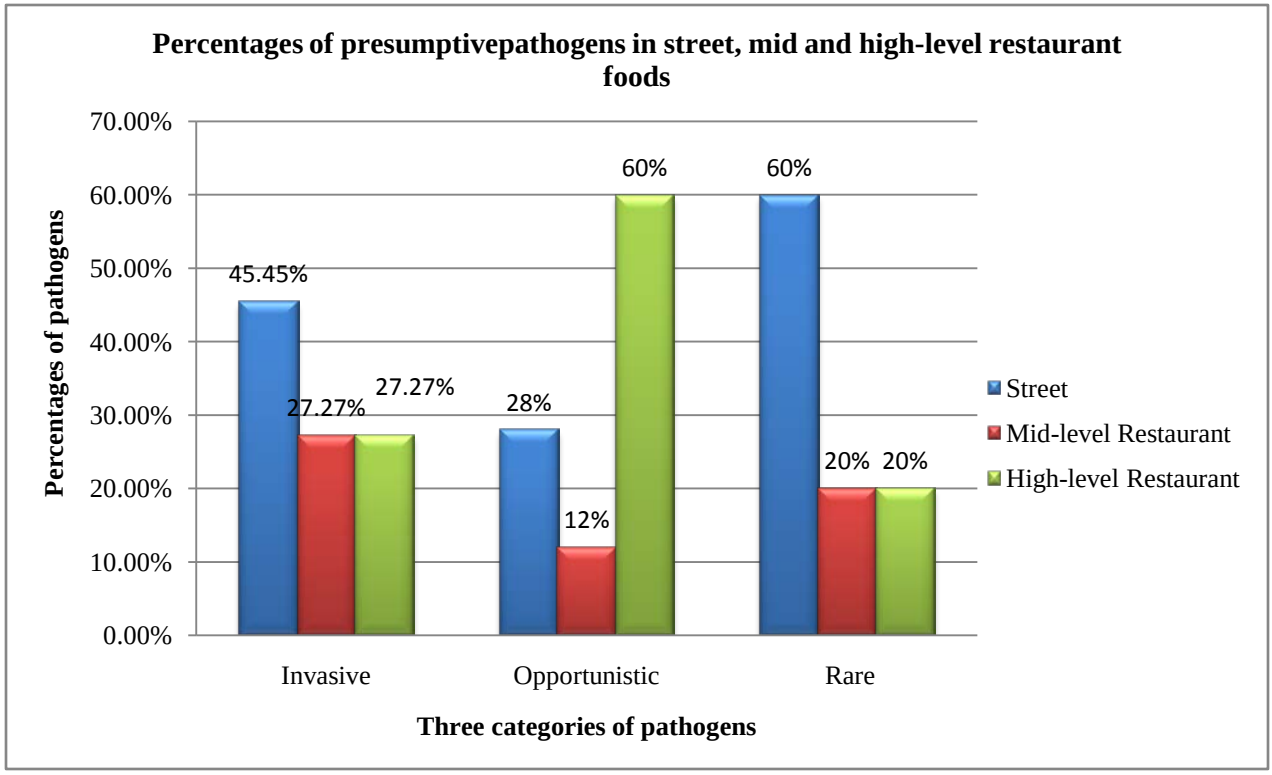

Figure-3.27: Column diagram presentation of pathogenic bacteria percentages.

The percentage of presumptive invasive pathogenic bacteria in street and high-level restaurant foods was higher than mid-level restaurant foods, which are 45.45% and 60% more than double and triple of the percentages of mid-level foods microbial load. However opportunistic pathogen percentages were higher in high-level restaurants and lower in street and mid-level restaurants, 28% and 12% respectively. Rare pathogens were present in 60% in street food and 20% in mid and high-level restaurant foods.

3.11. Percentages of specific presumptive pathogens

In this section presumptive invasive, opportunistic and rare pathogens are briefly described.

3.11.1 Percentage of presumptive invasive bacterial pathogen

The pathogen with unusual invading capability of human body parts are invasive pathogen. Invasive pathogens were identified from street, mid and high-level restaurant foods and there percentages are different in different collection location.

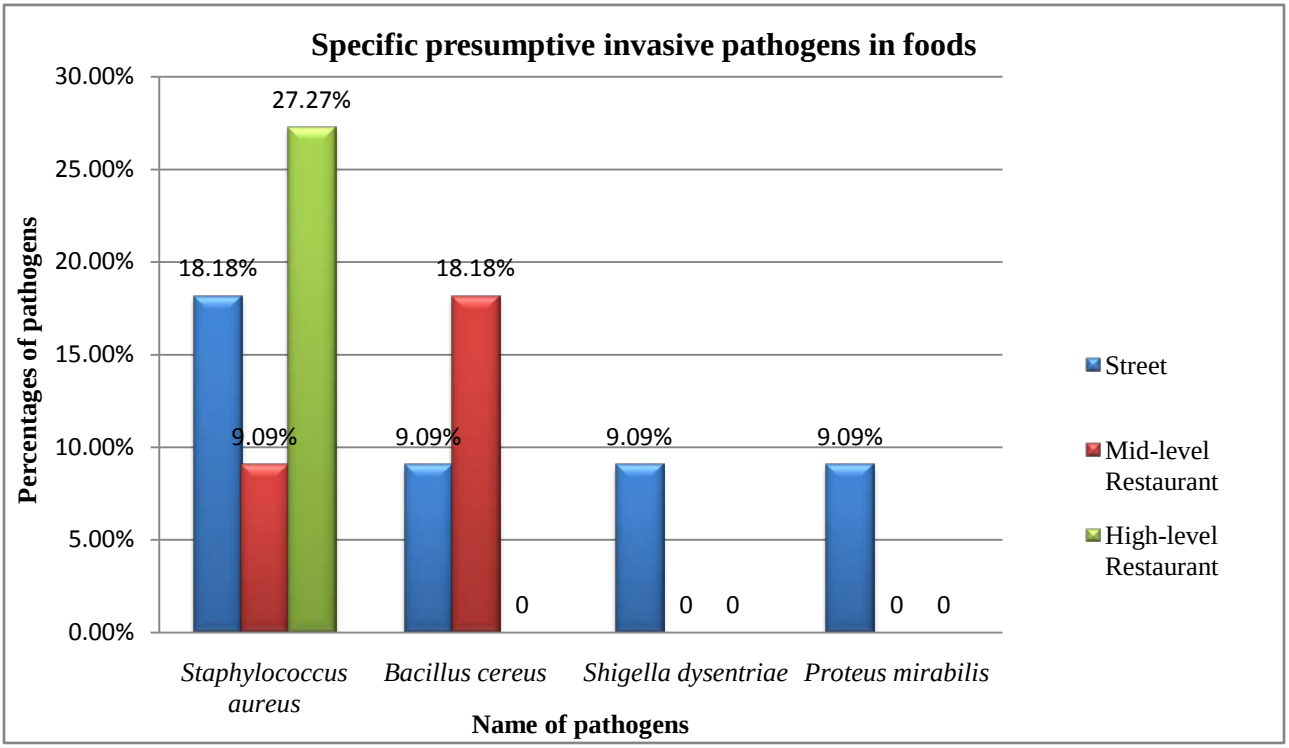

Figure-3.28: Percentages of specific invasive pathogens in street, mid and high-level restaurant foods.

Total four presumptive invasive pathogens were isolated from street, mid and high-level restaurants but not all of them were present in all food items some were present in street food, some in mid-level restaurant foods some are in high-level restaurant foods. *Shigella dysentriae* and *Proteus mirabilis* were isolated only from street foods at same percentage 9.09%. *Bacillus cereus* isolated from street and mid-level restaurant foods in 9.09% and 18.18% respectively from ruti, cake of mid-level restaurants and rice of street side stall. *Staphylococcus aureus* the only bacteria which was isolated from street, mid and high level restaurant foods in following percentages 18.18%, 9.09% and 27.27%. *S. aureus* was present in high percentage in high-level restaurant foods compared to street and level food.

Table-3.17: Presumptive invasive pathogens and their precise detail.

Invasive pathogen	**Disease**	**Where found**	**Cause of food contamination**
Staphylococcus aureus	superficial skin lesions such as boils, styes and furuncules, pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections, toxic shock syndrome	Human & animal skin, ear, mucous membrane, ubiquitous	Unhygienic food processing
Bacillus cereus	Diarrhea, stomach cramp, vomiting.	Soil, human and animal blood, animal carcasses and products	Food contamination by soil. Food handled by infected one.
Shigella dysentriae	'Shigellosis' bloody diarrhea, fever, stomach cramp	Contaminated water and food	Contaminated water source or food source
Proteus mirabilis	Urinary tract infection, kidney stone, kidney infection.	Soil, water and human intestinal tract.	Contaminated water source or food source

3.11.2 Percentages of specific presumptive opportunistic pathogens

Opportunistic pathogens are so called because it infects human when get opportunity to get in contact with any wound, diseased or surgery body parts of human. This microorganisms significantly pathogenic for infants as their immune system is not totally developed. Different type opportunistic pathogens were isolated and identified from street, mid and high-level restaurant foods. Percentages of these pathogens are presented below (Fig.-3.29),

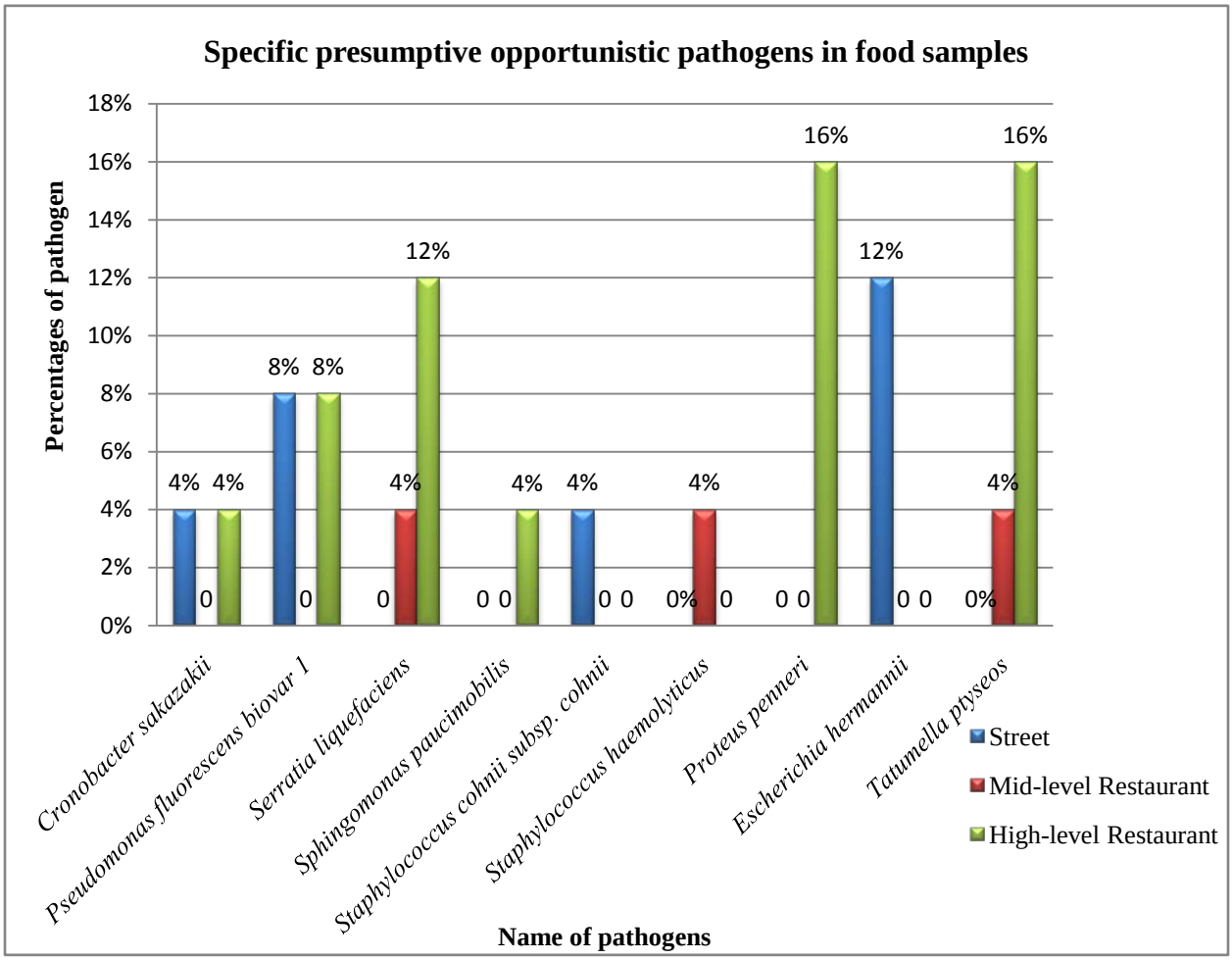

Figure-3.29: Percentages of specific opportunistic pathogens in street, mid and high-level restaurant foods.

In above Fig.-3.29 total nine presumptive opportunistic bacterial pathogens were isolated and identified from street, mid and high-level restaurant's food samples. Each pathogen was found in high-level restaurant foods with more or less percentages except *Staphylococcus cohnii* subsp. *cohnii* and *Staphylococcus haemolyticus*. *Tatumella ptyseos* is present in 16% and 4% in high and mid level restaurants. *Pseudomonas fluorescens* biovar 1 present in 8% in street and high

level restaurant foods and *Serratia liqefaciens* in 4% and 12% in mid and high level restaurant foods respectively. However *Cronobacter sakazakii* is present in 4% in street and high level restaurant.

However, three other bacteria *Staphylococcus haemolyticus, Staphylococcus cohnii* subsp. *cohnii, Sphingomonas paucimobilis* were found in relatively less percentage 4% in mid-level, street and high-level restaurant foods respectively. *Proteus penneri* and *Eshcherichia hermannii* were present in 16% and 12% in high and mid level restaurant foods. *Serratia liquifaciens* and *Tatumella ptyseos* were found in rice, cake and biscuit of high level restaurants. *Cronobacter sakazakii* was isolated and identified from rice and sugarcane juice of high-level restaurant and street level dal sample. *Proteus penneri* was isolated from 'dal' food sample, which is liquid food so the microbial load is generally higher. *Staphylococcus haemolyticus* isolated from mid-level restaurant's laddu sample. *Staphylococcus cohnii* subsp. *cohnii* isolated from street side biscuit sample and *Sphingomonas paucimobilis* isolated from high-level restaurant's biscuit sample.

Table-3.18: Presumptive opportunistic pathogens and their precise detail.

Opportunistic pathogen	Disease	Where found	Cause of food contamination
Cronobacter sakazakii	Invasive infections with high rate in neonates (meningitis, necrotizing enterocolitis & sepsis)	Human skin, plants, soil, water, sewage, clinical specimens	Unhygienic food processing
Pseudomonas fluorescens biovar 1	bacteremia	Oil, water, rarely clinical sample	From contaminated water
Serratia liqfaciens	Osteomyelitis, peritonitis, pneumonia, keratitis, conjunctivitis, urinary tract infections, sepsis, wound infections, nosocomial infections	Water, soil, foods, human & clinical specimens, mainly from wounds & the respiratory tracts Clinical specimens (blood, sputum, wounds, gallbladder, eye, abscess, throat),feces	Untidy food handling
Sphingomonas paucimobilis	nosocomial infections, septic arthritis and osteomyelitis	Soil, water	From contaminated water
Staphylococcus cohnii subsp. *cohnii*	Catheter related bacteremia	Human skin, hospital environment	From infected individual.
Staphylococcus haemolyticus	Localized and systemic infection	Skin, the axillae, perineum, and inguinal areas	From infected individual.
Tatumella ptyseos	Infection in immunocompromised, urinary tract infection, bacteremia	Human& animal specimen, respiratory tract, sputum, rarely from blood	Unhygienic food processing
Proteus penneri	Nosocomial infections of urinary tract and open wounds	Urine and stool	Urine or fecal contamination during handling
Escherichia hermannii	Septicemia, diarrhea	blood, stool and wound culture	From infected individual

3.11.3 Percentages of specific presumptive rare pathogens

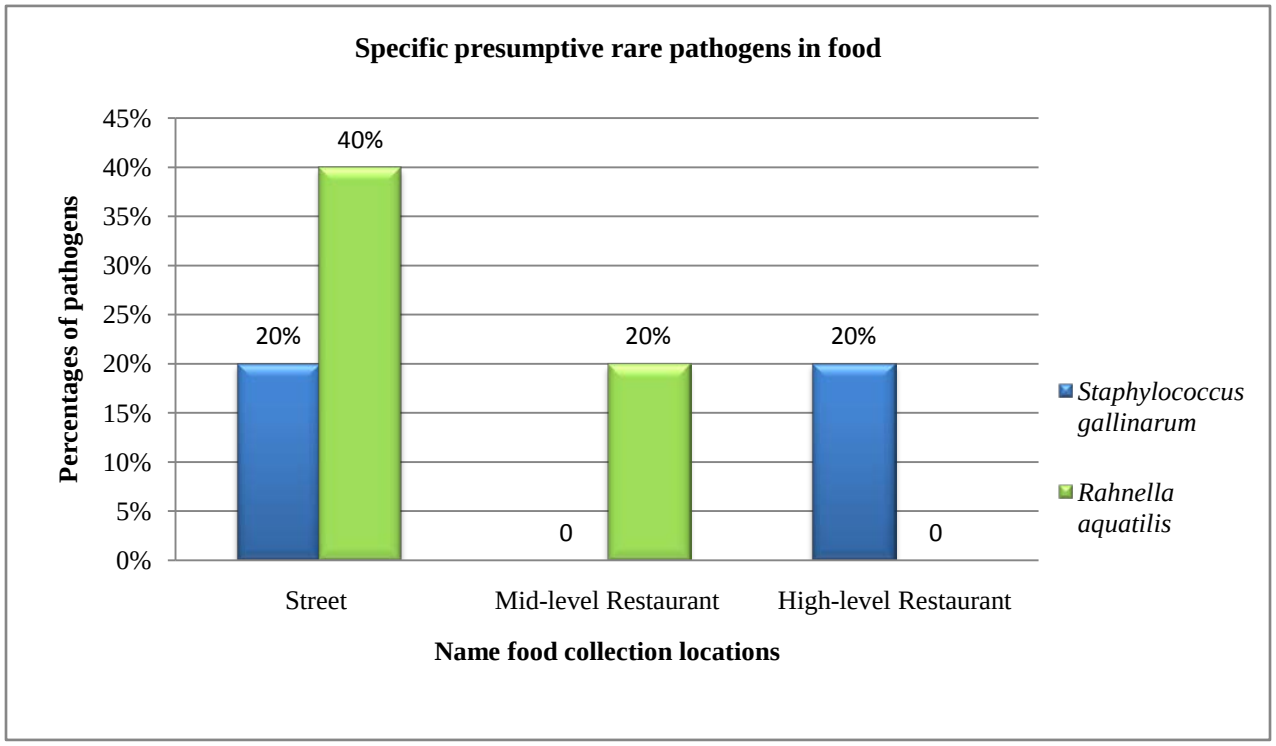

Figure-3.30: Percentages of specific rare pathogens in food.

Rare pathogens are not usually normal pathogen, it infect human in some exceptional condition, like immune compromised people, infants, aged people. A healthy person's immune system can inhibit this type of pathogens. Two types of presumptive rare pathogens such as *Staphylococcus gallinarum* and *Rahnella aquatilis* were isolated and identified from food samples of street, mid and high-level restaurants (Fig.-3.30). *Staphylococcus gallinarum* was identified from street and high-level restaurant foods at 20% and *Rahnella aquatilis* at 20% from mid-level and high-level restaurant foods. *Rahnella aquatilis* and *Staphylococcus gallinarum* both were found in street foods 40% and 20% respectively.

3.12 Antibiotic susceptibility test for specific pathogenic bacteria

Total eight isolates of bacteria were selected for antibiotic susceptibility test. Sixteen antibiotics were analyzed for each type of bacteria to see the sensitivity and resistance toward antibiotics. In this study eight isolates individually were tested one time for sixteen types of antibiotics. Two tables are prepared according Gram negative and Gram positive bacteria and showed sensitivity, resistance and intermediate according to the clear zone diameter.

Some bacteria have shown no clear zone which means it was resistant to the specific antibiotic and some shown very small diameter of clear zone this also an indicator of antibiotic resistance. If clear zone diameter is larger than resistant diameter scale and less than susceptible diameter than this result called intermediate, which means the specific bacteria is neither resistant nor susceptible to the specific antibiotic. The clear zone diameter scale when matches the susceptibility diameter scale then the bacteria is sensitive that specific antibiotic. The standard diameter scale is organized as a chart by antibiotic manufacturing companies and the test outcome is then matched with this chart to interpret the result.

3.12.1 Antibiotic susceptibility of gram negative bacteria

Total five Gram negative microorganisms were analyzed for antibiogram test (Table-3.19). Most of them were resistant to macrolides and penicillins. *Serratia liquifaciens*,*Cronobacter sakazakii* and *Shigella dysentriae* were resistant cefuxitin (30µg); one type of cephalosporines. *Proteus pinneri* showed resistant to tetracyclines along with macrolides and penicillins except amoxicillin (penicillin) (30µg). Only *Escherichia hermanni* showed resistant to cefuroxime-sodium (30µg) along with erythromycin(15µg) (macrolides) and amoxicillin (30 µg),oxacillin (1µg) sensitive to antibiotics gentamicin(10µg), kanamycin(30µg),streptomycin(10µg) (aminoglycosides), ciprofloxacin(5µg), nalidixic acid (30µg), norfloxacin (10 µg), pefloxacin (25µg) (fluoroquinolones), tetracycline (30 µg), doxycycline (30 µg) (tetracyclines), nitrofurantoin(10 µg) (nitrofurans). *Serratia liquifaciens*, *Cronobacter sakazakii* and *Escherichia hermanni* showed intermediate zone diameter against Streptomycin (10µg) one of aminoglycosides. *Cronobacter sakazakii*susceptible to gentamicin (10µg), kanamycin(30µg),streptomycin(10µg) (aminoglycosides), ciprofloxacin(5µg), nalidixic acid (30µg), norfloxacin (10 µg), pefloxacin (25µg) (fluoroquinolones), tetracycline (30 µg), doxycycline (30 µg) (tetracyclines), nitrofurantoin(10 µg) (nitrofurans), Chlorumphenicol (30

µg), sulfamethoxazole-trimethoprim(25µg) (sulfonamides). Among all selected antibiotic ampicillin was not tested for all bacteria.

3.12.2 Antibiotic susceptibility of gram positive bacteria

Bacillus cereus (Table-3.20) resistant to cephalosporines, ampicillins and sulfonamides and showed sensitivity to following antibiotics gentamicin(10µg), kanamycin(30µg),streptomycin(10µg) (aminoglycosides), ciprofloxacin(5µg), nalidixic acid (30µg), norfloxacin (10 µg), pefloxacin (25µg) (fluoroquinolones), erythromycin(15µg) (macrolides), tetracycline (30 µg), doxycycline (30 µg) (tetracyclines), nitrofurantoin(10 µg) (nitrofurans). *Staphylococcus haemolyticus* resistant to nalidixic acid(30µg), plefloxacin (25µg), ampicillin(10µg) and sensitive to aminoglycosides, cephalosporines, ciprofloxacin(5µg), amoxicillin (30 µg), oxacillin (1µg), tetracycline (30 µg), doxycycline (30 µg) (tetracyclines), sulfamethoxazole-trimethoprim(25µg) (sulfonamides).

Table-3.19: Antibiogram result for gram-negative bacteria.

Antibiotic classification	**Aminoglycosides**			**Cephalosporin**		**Fluoroquinolones**				**Macrolides**	**Penicillins**			**Tetracyclines**		**Nitrofurans**	**Sulfonamides**	**Chlorumphenicol**
Name of Gram-negative bacteria	Gentamicin(10µg)	Kanamycin(30µg)	Streptomycin(10µg)	Cefuroxime sodium(30µg)	Cefuxitin(30µg)	Ciprofloxacin(5µg)	Nalidixic acid (30 µg)	Nofloxacin(10 µg)	Pefloxacin (25µg)	Erythromycin(15µg)	Amoxicillin (30µg)	Ampicillin (10µg)	Oxacillin(1µg)	Tetracycline (30 µg)	Doxycycline (30 µg)	Nitrofurantoin(10 µg)	Sulfamethoxazoletrimethoprim(25µg)	Chlorumphenicol (30µg)
Proteus pinneri 91% (30%)	**S**	**S**	S	**S**	**S**	**S**	**S**	**S**	S	**R**	**S**	NA	**R**	**R**	**R**	**S**	**S**	**S**
Serratia liquifaciens 92% (32%)	**S**	**S**	I	**S**	**R**	**S**	**I**	**S**	S	**R**	**R**	NA	**R**	**S**	**S**	**S**	**S**	**R**
Serratia liquifaciens 91% (30%)	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**	S	**R**	**R**	**R**	**R**	**S**	**I**	**S**	**S**	**S**
Cronobacter sakazakii 92% (32%)	**S**	**S**	**I**	**S**	**R**	**S**	**S**	**S**	**S**	**R**	**R**	NA	**R**	**S**	**S**	**I**	**S**	S
Escherichia hermanni 92% (32%)	**S**	**S**	**I**	**R**	**S**	**S**	**S**	**S**	I	**R**	**R**	NA	**R**	**S**	**S**	**S**	**I**	S
Shigella dysentriae 76% (32%)	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**R**	**I**	NA	**R**	**S**	**R**	**S**	**S**	S
S= Sensitive, R= Resistant, I= Intermediate, NZ=No zone, NA= Not available																		

Table-3.20: Antibiogram result for gram-positive bacteria.

Antibiotic classification	**Aminoglycosides**			**Cephalosporin**		**Fluoroquinolones**				**Macrolides**	**Penicillins**			**Tetracyclines**		**Nitrofurans**	**Sulfonamides**	**Chlorumphenicol**
Name of Gram-Positive Bacteria	Gentamicin(10µg)	Kanamycin(30µg)	Streptomycin(10µg)	Cefuroxime-sodium(30µg)	Cefuxitin(30µg)	Ciprofloxacin(5µg)	Nalidixic acid (30 µg)	Norfloxacin (10 µg)	Pefloxacin (25µg)	Erythromycin(15µg)	Amoxicillin (30µg)	Ampicillin (10µg)	Oxacillin (1µg)	Tetracycline (30 µg)	Doxycycline (30 µg)	Nitrofurantoin(10 µg)	Sulfamethoxazole-trimethoprim(25µg)	Chlorumphenicol (30 µg)
Bacillus cereus 95% (22%)	**S**	**S**	**S**	**R**	**R**	**S**	**S**	**S**	S	**S**	**R**	**R**	**R**	**S**	**S**	**S**	**R**	R
Staphylococcus haemolyticus 80% (24%)	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**I**	R	**I**	**S**	R	**S**	S	**S**	**I**	S	R
	S= Sensitive, R= Resistant, I= Intermediate, NZ=No zone, NA= Not available																	

3.13 Some biochemical tests for seven selected isolates

Numbers of isolates were large therefore seven specific isolates were selected for more biochemical tests for further confirmations. Heamolysis tests were performed in sheep blood agar and three types of heamolysis were observed such as α-heamolysis, where blood cells were partially broken therefore the red color media converted into grayish-green after 24 hours of incubation. *Serratia liquifaciens, Shigella dysentrae, Cronobacter sakazakii, Proteus penneri* were showed partial blood cell lysis or α heamolysis in blood agar. If the blood cells are completely lysed a clear zone appeared surrounding the microbial growth and this type of blood cell lysis of blood agar media called β heamolysis. *Bacillus cereus* and *Eshcherichia hermanni* have shown β heamolysis. If blood cells are not lysed then it is called γ (gamma) lysis. *Staphylococcus haemolyticus* has shown gamma haemolysis in sheep blood agar.

Some bacteria are able to lyse starch by producing amaylase enzyme. In this study *Bacillus cereus* digest starch by amaylase enzyme and remaining six bacteria were negative for breaking the starch. If starch is lysed after incubation with specific bacteria culture a clear zone will appeared after adding iodine solution.

Protease is another enzyme produced by some bacteria and able to break down proteins. In this study milk agar was used to observe the caseinase (milk protein breaking protein) enzyme producing bacteria. A clear zone will appear if the caseinase breaks casein. *Bacillus cereus, Staphylococcus haemolyticus* and *Eshcherichia hermanni* showed caseinase positive activity in milk agar media after 24 hours of incubation and produced clear zones. *Serratia liquifaciens, Shigella dysentrae, Cronobacter sakazakii, Proteus penneri* were negative for caseinase activity.

Egg yolk agar was used to observe the lecithinase activity of bacteria. Egg yolk contain lecithin a protein and lecithinase a lipase enzyme can break down this protein and produce opaque halo zone surrounding the microbial growth. *Bacillus cereus* showed halo opaque zone after 24 hours of incubation and remaining bacteria did not give any opaque halo zone.

Another proteinase test has been done, which was gelatin liquefaction; gelatinase enzyme lyse the gelatin protein and then it takes liquid form. All isolates shown negative result for gelatinase activity. In next section a table-3.21 was prepared according to above tests results and also with resistant antibiotic name for each individual bacterium.

Table-3.21: Result of further biochemical tests and name of resistant antibiotics for specific bacteria.

Name of Gram-negative bacteria	**Resistant to**	**Haemolysis**	**Starch hydrolysis**	**Gelatin hydrolysis**	**Caseinase**	**Lecithinase**
Proteus penneri 91% (30%)	• Erythromycin (15µg) • Oxacillin (1µg) • Tetracycline (30 µg) • Doxycycline (30 µg)	α	-	-	-	-
Serratia liquifaciens 92% (32%)	• Cefuxitin (30µg) • Erythromycin (15µg) • Amoxicillin (30µg) • Ampicillin (10µg) • Oxacillin (1µg)	α	-	-	-	-
Cronobacter sakazakii 92% (32%)	• Cefuxitin (30µg) • Erythromycin (15µg) • Amoxicillin (30µg) • Oxacillin (1µg)	α	-	-	-	-
Escherichia hermanni 92% (32%)	• Cefuroxime-sodium (30µg) • Erythromycin (15µg) • Amoxicillin (30µg) • Oxacillin (1µg)	β	-	-	+	-
Shigella dysentriae 76% (32%)	• Cefuxitin (30µg) • Erythromycin (15µg) • Doxycycline (30µg) • Oxacillin (1µg)	α	-	-	-	-
Name of **Gram-positive bacteria**						
Bacillus anthracis 95%(22)	• Cefuroxime-sodium(30µg) • Cefuxitin(30µg) • Amoxicillin (30µg) • Ampicilline (10µg) • Oxicillin (1µg)	β	+	-	+	+
Staphylococcus haemolyticus 80% (24%)	• Nalidixic acid (30 µg) • Pefloxacin (25µg) • Ampicillin (10µg)	γ	-	-	+	-

Chapter 4

Discussion

4.1. Quantitative result

Based on this research study, it has been observed that, total viable count and enteric pathogen and coliform count of foods of street and high-level restaurants were higher than mid-level restaurant foods. This is due to street side dust, unhygienic food handling and processing, vehicle exhaust. However in high-level restaurant this high microbial count was due to temperature manipulation of foods, unawareness of food handling and processing, improper food storage. In case of mid-level restaurant's laddu the *staphylococci* count and fungi count were higher than street and high-level ones; this is because laddu of street was very dry and has low moisture and water activity and in case of high-level restaurant the hygienic level is highly maintained. Mid-level restaurant's laddus were made in unhygienic process; food processor may be not wearing gloves during laddu making process. The microbial load of sugarcane juice of high-level restaurants were higher than street side ones. This is due to the juicer not being properly cleaned and juice maker person's hygienic level being very low. Overall the microbial load of mid-level restaurant foods was lower than street and high-level restaurant foods.

4.2. Qualitative result

Three agar media HiCrome UTI agar, TCBS agar and XLD agar were used to observe the presence of certain microorganisms in food items. HiCrome UTI chromogenic agar used for identification of some Gram-negative and some Gram-positive bacteria which are responsible for urinary tract infection. The particular microorganisms are *Pseudomonas aeruoginosa, Enterococcus fecalis, E. coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis* which give colorless, blue-green (small), purple-magenta, golden yellow, blue-purple mucoid and light brown color respectively. Cleavage of chromogenic substrate with the help of enzymes produced by different bacteria causes the color change of colony [Himedia, HiCrome guide]. Escherichia coli give magenta colonies by cleaving the chromogenic substrate with the enzyme β-galactosidase. Blue to purple color colonies are produced by coliform bacteria due to the breakdown of chromogenic substrate. Nitrogenous and carbonaceous compound and other nutrients are provided by the peptic digest of animal tissue, beef extract. Presence of bile salt in the media inhibits the growth of Gram positive bacteria except *Staphylococcus aureus*, this make the agar more specific for Gram negative bacteria.

Thiosulfate Citrate Bile Salts Sucrose Agar is highly selective for *Vibrio* species isolation and the formula developed by Kobayashi and his colleagues, (Kobayashi *et al.*, 1963). The growth of intestinal flora suppressed at high pH of 8.5-9.5 of TCBS agar(Applied Microbiology, 1970). Different components allow the optimum growth and metabolic activities of *Vibrio* spp. such as, one percent sodium chloride and sodium thiosulfate provides a source of sulfur and in combination with ferric citrate to detect the production of hydrogen sulfide. The source of carbohydrate is sucrose also in combination with bromothymol blue and thymol blue indicators the *Vibrio* spp. give different color. Yellow-brown colonies are produced by *V. cholerae* biotype Eltor by fermenting sucrose and pH shifting. Light bluish colonies are produced by *V. parahaemolyticus* (*Applied Microbiology, 1970*). Small yellow colonies are produced by certain species of *Proteus* and enterococci which are easily distinguishable. In this study most of the colonies on TCBS were yellow, brownish-yellow also green colonies were also observed. After biochemical tests it was observed that almost all were non *Vibrio* spp. but not *Vibrio cholera* varieties.

Xylose-Lysine-Desoxycholate agar (XLD) is selective medium for the isolation of salmonellae and shigellae from clinical specimens and foods.

For isolation and primary differentiation of *Salmonella* and *Shigella* from non-pathogenic bacteria the media relies on xylose fermentation, lysine decarboxylation and production of hydrogen sulphide. Almost all enteric bacteria are capable of fermenting xylose except *Shigella, Providencia* and *Edwardsiella. Shigella* can be identified due to the xylose negative reaction.

Salmonella spp. is differentiated from non-pathogenic xylose fermenter by decarboxylation of lysine, which by altering the pH to alkaline and mimicking the *Shigella* reaction but producing hydrogen sulphide differentiates it. Lysine-positive coliforms cannot revert the pH to alkaline condition due to high level acid produced by fermentation of lactose and sucrose and also non-pathogenic hydrogen sulphide producers do not decarboxylate lysine. To inhibit the growth of coliforms without decreasing the growth of *shigellae* and *salmonellae* sodium desoxycholate is incorporated into the medium. Sodium desoxycholate is incorporated as an inhibitor in the medium. The concentration used allows for the inhibition of coliforms without decreasing the ability to support *shigellae* and *salmonellae (*Taylor W. I. and Harris B., 1967*).*

4.3. Microbial load comparison of raw and different categories of cooked foods

In this study seven different types of food items were examined, but not all were cooked as one of the food items was sugarcane juice which was raw food. Other six food items were cooked but they are categorized as moist cooked, dry cooked and sweet cooked food. Naturally, cooking temperature kill all possible microorganisms except some thermophile and spore forming bacteria. Cooked food items were contaminated after cooking due to improper food handling by food server. In this study it was observed that total viable count and *staphylococci* count of sugarcane juice was higher than other categories of cooked food items except enteric pathogen and coliform count and fungi count of liquid or moist cooked food. In liquid or moist cooked food items dal and rice are most of the time kept in a large bowl generally without covering the bowl; this is due to customer's pressure, stewards are not very conscious about the hygiene of foods. This is the common scenario of street and mid-level restaurants but in case of high-level restaurants, the kitchen is behind the main dining place so it is hard to observe. In this study, microbial load of high-level restaurant foods were higher than mid-level restaurant and street foods. This was may be due to unawareness of food processor's hygiene and being sold due to the brand name of high-level restaurant. For the same reason people are less conscious of the food quality, as it looks good and taste good. This fact is not applicable for all high-level restaurants because they have to keep their fame undisturbed. However mid-level restaurant food's microbial load status was better than street and high-level restaurants, and as customer pressure is higher than former two, so they have to keep maintain the food quality to attract customers come to the restaurant and only good food and good service can do that. All types people are the customer of mid-level restaurants, the food price is not as high as high-level restaurants and not as low as street side food shops, a reasonable price of foods have to maintain that customer can be happy with the service according to the price.

Water activity and moisture contents of food are other important factors which assist microbial growth in food at optimum temperature. However low water activity which means less water molecule available in food allow less growth of microorganisms. Dry cooked foods like cake, biscuit and allow less microbial growth inside the food, moreover sweet dry food also allow less microbial growth. In this study sweet dry food item gave less microbial load, as sweetener work as preservative.

4.4. Correlation and regression of water activity and microbial growth

The correlation between water activity and total viable count of high-level restaurant foods of Paribagh has shown moderate correlation and the value is 0.429 which is positive but less than 1. Value close to 1 indicates strong correlation. In the regression analysis the coefficient of determination value which is designated as r^2 is 0.184, which means 18.4% of the variation in viable count of the food sample is due to water activity of that food. The significance level (Fig. 3.18) of the t-test was 0.337 which is greater than 0.05, thus the null hypothesis is accepted; water activity is not a useful predictor of total viable count of food items. Whereas generally water activity is important factor to determine the total viable count or microbial growth, high water activity foods give high microbial load; but it is not always right as other factors are also determine the microbial growth, such as, pH, temperature, moisture content, food preservation condition etc. In this study raw food sugarcane juice of high-level restaurants have shown low viable total count compared to other cooked liquid, semi-solid and solid food items. This was may be for the post operating steps of cooked foods, such as, hygiene level of food processor and steward, utensils and surrounding environments. However the total viable count of sugarcane juice from same restaurant has shown high value than previous sugarcane juice, this was may be because the machine which used to extract juice from sugarcane was not properly washed or fresh juice mixed with remaining juice which was previously prepared. Sugarcane juice is raw food, flies and other insects are attracted to the juice and also juicer machine as a result the microbes form insects and surrounding environments contaminate the juice. Thus freshly prepared juice when mixed with previous juice gets contaminated. In the first sugarcane juice sample which shown less viable count, may be because the machine was washed and no prior juice was prepared by this juicer and also the person worn a hand gloves to prepare juice. Some opportunistic pathogens were isolated from rice, ruti and laddu having less water activity.

4.5. Mean comparison of total count of high-level restaurant foods ofParibagh and Kawran bazaar (T-test)

T-test was performed to compare means of total viable count of high-level restaurant foods of Paribagh and Kawran bazaar and also considered as group one and group two respectively.

Leven's Test for T-test was applied to determine whether there is any variability between total viable count of the high level restaurant foods of Kawran bazaar and Paribagh area.Means of two groups were 5.90 (Paribagh) and 6.40 (Kawran bazaar) respectively. According to this T-test (two-tailed) result, the significance level was 0.622 indicating that there is no significance difference between two groups of means.

4.6. Analysis of total count variation among street, mid and high level restaurant foods (ANOVA)

Means of total viable count of street, mid-level and high-level restaurant foods of Paribagh were compared for their variances. Gabriel post hoc test was chosen in ANOVA test as this does not rely on homogeneity of variances, because in this study sample size were not equal; sample number was seven for both street and high-level restaurants however, six food samples were analyzed in mid-level restaurant, as sugarcane juice was not available in mid-level restaurant. Significance level of variance of means between groups such as, street, mid-level restaurant and high-level restaurant (each considered as group) was 0.728 indicating that there was no statistical significance difference among three different groups. Moreover in multiple comparison test or post-hoc test was done and there was no significance difference among three groups as each significance level is around 0.9 greater than 0.05. Beside in this test the mean of total viable count of mid-level restaurant 4.84 which is lower than the means of street food 5.22 and high-level restaurant foods 5.63. A plot presentation in figure-25 also has shown a downward of line for mid-level restaurant foods' mean of total viable count from street foods' total viable count mean and again the gradual upward movement for high-level restaurant foods' mean. However, the differences among these means were not statistically significant.

4.7. Biochemical test results of isolates according to ABIS software

ABIS has shown most probable four bacteria and among those two highly probable bacteria were put into biochemical test chart in the result chapter of this thesis. The genera were approximately same but the species were not, more specific biochemical test are required to confirm the species.

According to this result further tables, chart and diagrams were prepared for categorization of pathogens and non-pathogens. In pathogens three categories were done such as, invasive pathogen, opportunistic pathogen and rare pathogen. Some invasive and opportunistic pathogens were selected for further biochemical test for more confirmation. These pathogens were selected according to the severity, frequency, occurrence, of diseases.

4.8. Pathogens from food samples: Percentages of pathogens and non-pathogens

As mentioned previously in the result section that ninety five isolates which were biochemically tested and among them 43% were pathogens and 57% were non-pathogens. Pathogens were categorized into three different groups, such as, invasive, opportunistic and rare pathogens and their percentages were 27%, 61% and 12% respectively. Some bacteria are usually not considered as pathogens as they are opportunistic or rare pathogens, but in this study all types of pathogens were counted. Opportunistic pathogens were present at high percentage in foods compared to invasive and rare pathogens. Contamination of food items with soil, water, infected individuals handling, germs from individuals' belongings, poor surrounding environmental condition during food processing, unhygienic food serving to the customer, use of unclear utensils etc. cause food contamination. Healthy individuals are usually not susceptible to opportunistic pathogens as their immune systems are strong enough to fight against opportunistic and rare pathogens. These three categories of pathogens were found in street, mid-level and high-level restaurant foods and their percentages are presented in column graph in result section. It was observed that half of invasive pathogens were isolated from street foods and one fourth from mid-level restaurants another one fourth from high-level restaurants (Figure-3.27). Total four types of isolates were identified and assumed as invasive pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Shigella dysentriae* and *Proteus mirabilis* and the contamination of food occurred from soil, water, human skin, ear, mucous membrane of infected people. Street side slum like shops are not hygienic place for selling food, because there are lots of sources for food contamination, like it is a open place where road side dust, vehicle exhaust, air-borne germs, germs from sneezing, coughing, spitting, road side defecating of passers-by, road side open dustbins are located . However opportunistic pathogens were present at 60% in high-level restaurant foods but 28% in street and 12% mid-level restaurant foods respectively. Total nine types of opportunistic pathogens such as, *Cronobacte rsakazakii*, *Pseudomonas fluorescens*

biovar 1, *Serratia liqfaciens*, *Sphingomonas paucimobilis*, *Staphylococcus cohnii* subsp. *cohnii*, *Staphylococcus haemolyticus*, *Tatumellaptyseos*, *Proteus penneri* and *Escherichia hermannii* were identified from all food items. *Tatumella ptyseos* contamination of food may occur from infected individual's skin, sputum, urine and also *Proteus penneri* contamination occur due to fecal contamination of food. However *Cronobacter sakazakii* and *Escherichia hermannii* contamination may be present due to soil or water contamination, from skin, clinical specimens of human. Unawareness of food processing hygiene, grimy utensils, steward's poor personal hygiene, and storage of the remaining cooked food items with raw food items and serving the previous food without proper processing etc. cause microbial contamination of high-level restaurant foods. Simply it is assumed that in high-level restaurants hygiene is properly maintained, not all but most of them are reluctant of proper food processing, serving, storing; may be due to the fame of the restaurant and less competition compared to mid-level restaurants. Rare pathogens are present at 60% in street food, 20% each in mid and high-level restaurant foods. Usually street foods are prone to microbial contamination and other types of contamination and presence of rare pathogen like *Rahnella aquatilis* and *Staphylococcus gallinarum* also occur beside invasive and opportunistic pathogen contamination.

4.9. Microbial load of street food

In this study four types of microbial loads were observed such as, total viable count (TVC), coliform and enteric pathogen count (CEC), *staphylococci* count (SC) and fungi-yeast count (FYC). In total seven food items were examined for microbial load i.e. from street side, mid-level restaurant and high-level restaurants of Paribagh and Kawranbazar.

In Paribagh street side, among seven food items rice, dal and sugarcane juice showed four categories of microbial loads and remaining four food items cake, laddu, biscuit and ruti showed three types microbial loads except ruti which showed total count and staphylococci count but one thing is common among these four types of food which is coliform count was not observed. The absence of coliform count may be for less moisture content and water activity. As previously mentioned that water activity (Aw) is unbound water molecules available in food and moisture content is equilibrium humidity of the food. Other reasons for no coliform count was may be due cooking temperature of food and also less handling of these food items compared to former three food items such as rice, dal and sugarcane juice. Rice and dal are regularly consumed by people three or two times a day as main meal however sugarcane juice sold raw in street side consumed as cold fruit drink. Rice is kept in a large bowl from where cook serve with bare hand or with a large spoon or small plate, dal also is kept in a large bowl and served by spoon or small mug. Sometimes the bowls are covered with a net cover or by cloth but most of the time they are not covered, so flies, road side dust, germs from sneezing, coughing of people and dustbin contaminate foods. In case sugarcane juice, peeled up sugarcane stacked on cart beside the juicer. Two adjacent steel rollers through which sugarcane is passed and the roller is operated by motor through which peeled sugarcane passed and moving roller press sugarcane and juice is collected in a pot. People drink this juice street side, vendor use few glasses for many customers and the glass is washed in same water kept in small large pot. Crushed ice is used to cool the juice but source of water for ice is uncertain, the ice used for the preservation of fish is also used for juice. Road side dust, exhaust gas from vehicles, air borne germs, germs of people's sneezing, coughing contaminate the juicer and also the juice. However biscuit kept in a container and laddu, cake are kept in transparent plastic bag which hanged from a hook so that customer can see the food items and buy them. Ruti made kept in large plate and served to the customer with bare hand.

4.10. Opportunistic, invasive and rare pathogens

Staphylococcus cohnii Gram positive cocci categorized as opportunistic pathogen. In this study it was isolated from mid-level restaurant foods. It is most concerning bacteria of different States of USA. In Arizona and California State it has been reported that *S. cohnii* causes skin infections, food poisoning, endocarditis, urinary tract infections and septicemia. The strain of this staphylococci produces hemolysin and cytotoxins (PawelMak et al.,).A fatal meningitis of 63-year-old male due to *S.cohnii* infection (Okudera *et al.*, 1991). The manipulation of food processing, refrigeration temperature allows the growth of *S.cohnii* or competing microflora has been destroyed or inhibited by cooking (Richard lawley, Laurie Curtis, Judy Davis; The Food Safety Hazard Guidebook).

Staphylococcus heamolyticus is another second most opportunistic pathogenic bacterium after *Staphylococcus epidermis*. *S. hemolyticuswas* isolated from mid-level restaurant food laddu. This Gram positive cocci is coagulase negative staphylococci (CoNS) found in human skin, inguinal, axillae, perineum areas. This staphylococcus produces hemolysin protein, enterotoxins and lipase. This bacterium can cause meningitis, skin or soft tissue infections, prosthetic join infections, or bacteremia. It is resistant to methicillin, gentamycin, erythormycin, and uniquely among staphylococci, glycopeptide antibiotics [Falcone. M.*et al.*, 2007].However in this study this presumptive strain was resistant to ampicillin, plefloxacin, nalidixic acid, chlorumphenicol, intermediate sensitive to erythromycin and full sensitive to oaxacillin, tetracycline, kanamycin, gentamycin.

Staphylococcus aureus is normal skin flora of 25% healthy human and it does not cause diseases unless mixed with foods. *Staphylococcus aureus* is significant among *staphylococcus* genus as it can produce different types of toxins which are heat resistant .These enterotoxins cause diseases like stomach cramp, nausea, retching, vomiting, diarrhea etc. after one to six hours of consumption. *Staphylococcus* can be found in unpasteurized milk, cheese and also in salty food items as it can tolerate high concentration of salt. Food poisoning can occur in several ways one of which is direct handling of food items after cooking or no cooking (Centers for Diseases Control and Preventions, staphylococcal food poisoning).

Other rare *Staphylococci* is *Staphylococcus gallinarum* isolated from skin of poultry, Japanese quail, silk worm larvae, cat skin, human clinical samples(**StoicaCostin. *Regnum Prokaryotae*, http://www.tgw1916.net, accessed on Mar 2012**). It was isolated from hepatitis B infected patients' blood (Yu, D *et al.*,2008), eye infection (Tibra, N. K. *et al.*, 2009). This strain is non-haemolytic, coagulase negative. *Staphylococcus gallinarum*is found in ewe milk cheese with other *staphylococci* species.

Food contamination occurs due to unhygienic processing of food where food is open to animals like birds, cats or food processor handling the food after processing poultryitems without washing hands (María Camino García*et al.*).

Eshchirichia hermannii is a gram negative coliform bacterium previously called enteric group 11. Generally *E. hermannii*can be isolated from clinical sample like sputum, stool, wounds etc. but few strains were isolated from foods in United States (Don J. Brennner*et al.*, 1981). According to Hernandez et al., 1998 *E. hermannii* isolated from soils of oil refinery which suggests that this organism can survive extreme environmental conditions.

It has been reported that *E. hermannii* show positive result for indole, methyl-red test, mucate, gas and acid from D-glucose, maltose, D-xylose, L-arabinose, D-mannitol and negative result for Voges-Proskauer, Simmon's citrate, H_2S, urea, phenylalanine deaminase and hydrolysis. Antibiotics resistant to were penicillin, ampicillin, carbicillin and sensitive to colistin, nalidixic acid, sulfadiazine, gentamicin, streptomycin, kanamycin, tetracycline, chlorumphenicol, cephalothin. In this study this presumptive strain of *E. hermannii* has shown positive for indole, methyl-red test, acid and gas from D-glucose, lactose and acid from sucrose and negative result for Voges-Proskauer, Simmon's citrate, H_2S, urea, gelatin hydrolysis. *Escherichia hermanni* showed resistant to cefuroxime-sodium along with erythromycin (macrolides) and amoxicillin,oxacillin sensitive to antibiotics gentamicin, kanamycin, streptomycin (aminoglycosides), ciprofloxacin, nalidixic acid, norfloxacin, pefloxacin (fluoroquinolones), tetracycline, doxycycline (tetracyclines), nitrofurantoin (nitrofurans). It is an opportunistic pathogen and cause purulent conjunctivitis, periodontal lesions, septicemia, neonatal brain infections, meningitis, and wound infection.

Cronobacter sakazakii or ***Enterobacter sakazakii*** is a Gram-negative fatal opportunistic pathogen found in infant food formula, wide range of other food samples and environmental

samples (Ziad W Jaradat *et al.*, 2009). In this study it has been isolated from sugarcane juice and rice of street and high-level restaurant, dal of street side shop. These contaminations occur due to improper food processing, cooking, serving and use of untidy utensils. *Cronobacter* usually infects immune compromised individuals, like infants, older people, ill people etc. It causes sepsis, severe meningitis, seizures experience in infants. Long-term neurological problems occur due to consequences of meningitis such as, brain abscesses or infarcts, hydrocephalus or other serious complications. However in adult it causes urinary tract infection, wound infection, blood stream infection, colonization in respiratory tract by mechanical respiratory ventilation system. Sometime it remains asymptomatic inside the human body (Center for Disease Control, 2014). It has been reported that *C. sakazakii*is sensitive to ampicillin, cefotaxime, chlorumphenicol, gentamicin, kanamycin, polymyxin B, streptomycin, sulfomethoxazole, tetracycline, and resistant to sulfisoxazole, cephalothin (Maria Nazarowec-white, 1998). In this study this strain was found to be sensitive togentamicin, kanamycin, (aminoglycosides), ciprofloxacin, nalidixic acid, norfloxacin, pefloxacin(fluoroquinolones), tetracycline, doxycycline (tetracyclines), chlorumphenicol, sulfamethoxazole-trimethoprim (sulfonamides) and resistant to cefoxitin, erythromycin, amoxicillin and oxacillin and has shown intermediate sensitivity to nitrofurantoin (nitrofurans) and streptomycin (aminoglycosides). According to ATCC the biochemical characteristics of *cronobacter sakazakii* is citrate (+ve), hydrogen sulfide (-ve), urease (-ve), tryptophane deaminase (-ve), indole (-ve), Voges-Proskauer (-ve), gelatinase (-ve), however in this study the negative results were obtained from hydrogen sulfide, urease, indole, starch, lecithinase, gelatinase and positive for motility, citrate, methyl-red, Voges-Proskauer, catalase, nitrate, acid from dextrose, sucrose and lactose. The probability of this bacteria to be *Cronobacter sakazakii* is high i.e. almost 92% and based on 32% accuracy of the result to the ABIS software, it can be assumed that it is *Cronobacter sakazakii*.

Shigella dysentriae is a Gram negative, rod shaped, non-motile, non-spore forming pathogenic bacteria. *S. dysentriae* usually found in contaminated water, food, unhygienic environment like overpopulated area, malnourished people, poor waste management area; usually it survives poorly outside human body. In this study it was isolated from dal of street side stall. This bacterium generally gives negative result for oxidase, urease, motility, lactose, citrate and positive for catalase, and alkaline butt and acid slant. In this study this strain has shown negative result for dextrose, sucrose, lactose, indole, motility, citrate, hydrogen sulfide and positive for

methyl-red, nitrate, urease, Voges-Proskauer and acid butt and acid slant in TSI. In case of disc diffusion antibiogram test generally this bacteria is

resistant to co-trimoxazole, tetracycline, ampicillin, gentamicin, nalidixic acid, chloramphenicol (G. Wilson*et al.*, 2005). In this study presumptive *S. dysentriae* has shown resistance to cefoxitin, erythromycin, doxycycline and oxacillin and sensitive to gentamicin, kanamycin, (aminoglycosides), ciprofloxacin, nalidixic acid, norfloxacin, pefloxacin (fluoroquinolones), tetracycline, chlorumphenicol, sulfamethoxazole-trimethoprim (sulfonamides), erythromycin, amoxicillin. The similarities of biochemical test and antibiogram results are very low and the ABIS software probability was 76% and accuracy of results was 32% and it was not satisfactory. It may be other species of *Shigella* but not *S. dysentriae*. Further test is necessary for accurate identifications as this study is mainly focused on microbial load determination of street, mid-level and high-level restaurant foods.

Proteus penneriis a Gram negative, rod shaped, motile, facultative anaerobic bacteria, found in clinical specimens like blood, stool, pus, urine and it causes nosocomial infection of open wound and also infection of urinary tract, blood, abdominal wound, neck, ankle, and groin. One of its recognizing characters is it has a fishy smell. In this study *Proteus penneri* was isolated from dal of high-level restaurants. The contamination occurs due to improper food handling after cooking, use of untidy utensils or food is handled by infected individual. Generally this bacteria shows positive result for nitrate, catalase, ferment glucose with gas, sucrose, urease, hydrogen sulfide, haemolysis, swarming and negative result for oxidase, indole, lactose fermentation (Janak Kishore,2012). In this study this strain has also shown positive result for nitrate, catalase, and ferment glucose with gas, sucrose, urease, hydrogen sulfide, haemolysis, swarming and negative result for oxidase, indole, and lactose fermentation. It has been reported by Janak Kishore that this strain of *Proteus*is resistance to penicillin G, amoxicillin, cephalosporins (*i.e.*cefaclor, cefazoline, cefuroxime and cefdinir), oxacillin, most of the macrolids but naturally sensitive to aminoglycosides, carbapenems, aztreonam, quinolones, sulphamethoxazoleand co-trimoxazole. In this study *P.penneri* has shown resistance to erythromycin (macrolides), oxacillin, tetracycline, doxycycline and sensitive to gentamicin, kanamycin, streptomycin (aminoglycosides), ciprofloxacin, nalidixic acid, norfloxacin, pefloxacin(fluoroquinolones), chlorumphenicol, sulfamethoxazole-trimethoprim (sulfonamides), and amoxicillin. The

antibiogram result is almost same as that reported by Janakki shoreand Caroline Mohr O'Hara *et al.*, (2000). Thus the isolated bacterium was assumed to be *Proteus penneri*.

Serratia liquefaciens a gram negative rod isolated from street food, is usually found in environment and colonize on soil, water, plants, and the digestive tracts of the rhodents, insects, fish, and humans [Labbate*et al.*, 2004].Generally *S. liquefaciens* considered as non-pathogenic but some strains cause nosocomial infections, urinary tract infections, bloodstream infections, sepsis, pneumonia, meningocephalitis, and other slow growing infections and sometimes cause death(Dubouix A. *et al.*, 2005). Nosocomial infections occur due to unhygienic handling of medical equipment, medicines and other similar materials (Harnett *et al.*, 2001). High Pressure Processing (HPP) is used to inactivate *S. liquefaciens* in dry-cured ham (N. Belletti *et al.*, 2013). Moreover *Serratia* and *Proteus* were found to be present in meat processing industry (Stiles, 1981). *Serratia liquefaciens* is a major spoiling agent in fresh, cooked and cured- meat (Silla-Santos, 1996]. Unhygienic food handling causes *S. liquefaciens* food contamination. It has been reported in two papers that *S. liquefaciens* is resistant to oxacillin, amoxicillin, erythromycin, ampicillin, cefuxitin, nitrofurantoin, tetracycline, cefazolin (Ingo Stock et al., 2003) (Claudia Sala*et al.*, 2012). In this study presumptive *Serratia liquefaciens* resistant to oxacillin, ampicillin, amoxicillin,cefuxitin, and sensitive to nitrofurantoin, gentamicin, kanamycin, streptomycin (aminoglycosides), ciprofloxacin, nalidixic acid, norfloxacin, pefloxacin (fluoroquinolones), tetracycline, doxycycline (tetracyclines), nitrofurantoin (nitrofurans) was noted.

Bacillus cereus is a ubiquitous Gram positive rod, obligate aerobe or facultative anaerobe, found in soil, water and other environmental samples, clinical specimens, like blood, sputum- reported in North Carolina Memorial hospital in 1981 to 1985. This bacteria cause severe infections like endocarditis, sepsis, meningitis, endophthalmitis, and surgical wound infections (Cornord J.D. *et al.*, 1971, Cotton D.J. *et al.*, 1987; Davey *et al.*, 1987). It is a food poisoning bacteria and cause profuse watery diarrhea with abdominal cramp, rectal tenesmus, and occasionally fever, vomiting, malaise [Logan, N. A. *et al.*, 2006). In this study a motile and β-hemolytic bacillus was isolated and the biochemical results suggested most probable (88%) bacteria name *Bacillus cereus*with the help of ABIS. This *Bacillus* has shown resistance to amoxicillin, oxacillin, ampicillin, cefuxitin, cefuroxime-sodium, chlorumphenicol and shown sensitivity to gentamicin, kanamycin, streptomycin (aminoglycosides), ciprofloxacin, nalidixicacid, norfloxacin,

pefloxacin (fluoroquinolones), erythromycin, tetracycline, doxycycline, nitrofurantoin. It has been reported that this bacteria susceptible to aminoglycosides, ciprofloxacin, erythromycin and gentamicin (Logan, N. A. *et al.*, 2006; Murry, P. R. *et al.*, 2007; Topley & Willson's, 1998). In this study this *Bacillus cereus* strain was resistant to chloramphenicol and also citrate negative thus the probability of ABIS was 88% as *Bacillus cereus*. Further biochemical tests are needed to perform to ensure the species. Some time variable results were observed in same species of bacteria.

Rahnella aquatilisis a Gram-negative rod and rare pathogen found in water, soil, certain animals, like beetles, snails (Brenner *et al*., 1998), and isolated human clinical specimens (R.J. Martinez, J Bacteriol, 2012). It is also known as food contaminant (ABIS encyclopedia). Highest histamine levels in fish products cause *Rahnella aquatilis* contamination. *R. aquatilis* symbiotically grow in plant nodules for nitrogen fixation, the adhesin protein (pore forming protein)is very similar to Gram-negative pathogens' outer membrane protein. The pathogenecity is also reported, such as it has been isolated from patient blood cultures, respiratory washings, and wound cultures. Infections are also caused by this bacterium, such as bacteremia (renal infection), sepsis, respiratory infection, and urinary tract infection. Infective endocarditis has been developed with congenital heart diseases in 11-month-old girl (Matsukura H.*et al.*, 1996). In this study it has been isolated from mid-level restaurant and street side stores dal and street sugarcane juice in 91% and 88% respectively; both items are liquid, one is cooked another is raw. These food items are contaminated during food preparing, handling and serving by contaminated water, soil other available sources.

Chapter 5

Conclusion

5. Conclusion

The present research study has been carried out to, investigate and compare the microbial quality of foods collected from street side stalls, mid-level restaurants and high level restaurants. These collected foods were handled appropriately to preserve the food quality before lab testing to obtain accurate microbial quality and further investigative analysis. In addition to microbial quality analysis, further relevant studies have also been carried out to compare the street food with homemade food, restaurant food with homemade food, comparison among street, restaurant and homemade foods. Utmost precaution and standard protocols have been seriously maintained to carry out these lab tests and other relevant investigations.

Based on the results reported here, it can be said that, almost all studies have showed less microbial load in homemade foods compared to foods that were obtained from various street side vendors and restaurants although, certain foods obtained from high level restaurants showed less microbial load compared to street foods. All these results have been charted out appropriately in this research thesis for further investigative study and future works. Also various relevant comparison study and charts are detailed in this thesis to specifically highlight the microbial load in various food sources. High microbial load in street foods occur due to improper food handling, unhygienic food preparation and processing, cooking and storage at inappropriate temperature,. Government, consumer, food vendors, chef, stewards and all type people should be aware of food hygiene, public health, implications of consuming contaminated foods, causative diseases. 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.

Following points should be strongly observed and maintained for future work and further investigative study to improve the food quality, based on this research study on street side foods and restaurant foods:

1. Classification of available non-homemade foods based on their food quality and microbial loads.
2. Ensuring regular inspection and periodical check on these food preparation procedures.

3. Continuous lab test and analytical lab analysis to check the unwarranted presence of any new harmful agents in these foods to ensure food safety for consumers.

Chapter 6

Reference

Reference

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Appendices

Appendix-I

Different types of media used for count

1. Nutrient Agar (Himedia) Composition: gm/liter	
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.5
Yeast extract	1.5
Agar	15.00

Use:

For cultivation of less fastidious microorganisms, can be enriched with blood or other fluid

pH at 25^0 C 7.4±0.2

2. MacConkey Agar w/0.15% Bile salts, CV and NaCl (Himedia) Composition: gm/liter	
Peptic digest of animal tissue	1.5
Casein enzymic hydrolysate	1.5
Pancreatic digest of gelatin	17.00
Lactose	10.00
Bile salts	1.50
Crystal violet	0.001
Neutral red	0.03
Agar	15.00

Use:

For selective isolation and differentiation of coliform organisms and other enteric pathogen

pH at 25^0 C 7.1 ±0.2

3. Eosine Methylene Blue (Oxoid) Composition: gm/liter	
Peptone	10.00gm
Dipotassium phosphate	2.00
Lactose	5.00
Sucrose	5.00
Eosin yellow	0.14
Methylene blue	0.065
Agar	13.50

4. Mannitol Salt Agar Base(Himedia) Composition: gm/liter	
Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	75.00
D-Mannitol	10.00
Phenol red	0.025
Agar	15.00

Use:

For selective isolation of pathogenic *Staphylococci*.

pH at 25^0 C 7.4 ±0.2

5. Saburoud Dextrose Agar (Himedia) Composition: gm/liter	
Mycological peptone	10.00
Dextrose	40.00
Agar	15.00

Use:

For cultivation of yeasts, moulds and aciduric microorganisms

pH at 25^0 C 5.6 ±0.2

6. Thiosulfate-Citrate-Bile-Salts-Sucrose Agar (Difco™ BD) Composition: gm/liter	
Proteose Peptone	10.00gm
Yeast extract	5.00
Sodium thiosulphate	10.00
Sodium citrate	10.00
Oxgall	8.00
Sucrose	10.00
Sodium chloride	10.00
Ferric citrate	1.00
Bromo thymol blue	0.04
Agar	15.00

Use:

Recommended for the selective isolation and cultivation of *Vibrio cholerae* and other entero pathogenic Vibrios causing food poisoning.

pH at 25^0 C 7.4 ±0.2

7. Xylose-Lysine-Deoxycholate Agar (Himedia) Composition: gm/liter	
Yeast extract	3.00
L-lysine	5.00
Lactose	7.50
Sucrose	7.50
Xylose	3.50
Sodium chloride	5.00

Use:

For selective isolation and enumeration of *Salmonella typhi* and other *Salmonella* species.

pH at 25^0 C 7.4 ±0.2

Sodium deoxycholate	2.50
Sodium thiosulfate	6.80
Ferric ammonium	0.80
Phenol red	0.08
Agar	15.00

8. HiCrome UTI Agar (Himedia) Composition: gm/liter	
Peptic digest of animal tissue	15.00
Chromogenic mixture	26.80
Agar	15.00

Use:

Isolation of urinary tract infection pathogen.

pH (at 25^{o}C)

Enrichment Broth

a. Alkaline peptone Water (pH 8.4±0.2) Composition: gm/liter	
Peptic digest of animal tissue	10.0gm
Sodium chloride	10.0gm

Use:

For selective isolation of pathogenic *Vibrio* spp.

pH at 25^{0} C 7.4 ±0.2

Selenite broth (Difco) Composition: gm/liter	
Pancreatic Digest of Casein	5.0
Lactose	4.0
Sodium Selenite	4.0
Sodium Phosphate	10.0

Use:

For selective isolation of pathogenic *Salmonella* spp.

pH at 25^{0} C 7.4 ±0.2

Broth for Stock culture

Tryptone soya broth (Oxoid) Composition: gm/liter	
Tryptone soya broth	30gm
Glucose	5.0gm
Glycerol	100ml

1. Simmon's Citrate Agar (Oxoid) Composition: gm/liter	
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Ammonium phosphate	0.8
Sodium citrate tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.00

Use:

pH 7.5±0.2 at 25^{o} C

Microbial Simmon's citrate utilization test

2. Triple Sugar Iron Agar (Himedia) Composition: gm/liter	
Peptic digest of animal tissue	10.00
Casein enzymatic hydrolysate	10.00
Yeast extract	3.00
Beef extract	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium chloride	5.00
Ferrous sulfate	0.02
Sodium thiosulfate	0.30
Phenol red	0.024

Agar	12.00

3. Motility Indole Urea Agar (Oxoid) Composition: gm/liter	
Casein enzymic hydrolysate	10.00
Dextrose	1.00
Sodium chloride	5.00
Phenol red	0.01
Agar	2.00
40% Urea Solution	5ml

Use:

For detection of motility urease and indole production.

pH at 25^0 C 6.8 ±0.2

4. Methyl Red and Voges-Proskauer Broth Composition: gm/liter	
Peptone	7.0gm
Dextrose	5.0gm
Potassium phosphate	5.0gm

5. Nitrate Broth Composition: gm/liter	
Peptone	5.0gm
Beef extract	3.0gm
Potassium nitrate	5.0gm

6. Phenol Red Broths (pH 7.3)

6.2. Phenol Red sucrose Broth Composition: gm/liter	
Trypticase	10gm
Sucrose	5.0gm
Sodium chloride	5.0gm
Phenol red	0.018gm

6.2. Phenol Red Dextrose Broth Composition: gm/liter	
Trypticase	10gm
Dextrose	5.0gm

Sodium chloride	5.0gm
Phenol red	0.018gm

6.3. Phenol Red Lactose Broth Composition: gm/liter	
Trypticase	10gm
Lactose	5.0gm
Sodium chloride	5.0gm
Phenol red	0.018gm

Appendix II

Reagents

Kovac's reagent

1.25 gm of para-dimethylaminobenzaldehyde 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 with constant stirring to make 25 ml 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 dissolved by stirring. Distilled water was added to adjust the final volume to 25 ml.This solution was covered with aluminum foil and stored at 4^{o}C.

MacFarlane turbidity standard no.5

Sulfuric acid	0.18M
Barium chloride	0.048M
Distilled Water	1000ml

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 4^{o}C.

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-napthaylamine dissolved in 120ml of 5N acetic acid.

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°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