HYGIENE PRACTICES, MICROBIAL DIVERSITY AND DETECTION OF PATHOGENIC BACTERIA IN SOME READY-TO-EAT FOODS OF RAJSHAHI METROPOLIS

A Thesis Submitted for the Degree of Master of Philosophy in the Department of Botany, University of Rajshahi, Bangladesh

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DECLARATION

I do hereby declare that this thesis paper entitled "***HYGIENE PRACTICES, MICROBIAL DIVERSITY AND DETECTION OF PATHOGENIC BACTERIA IN SOME READY–TO-EAT FOODS OF RAJSHAHI METROPOLIS***" has been originally carried out by me under the guidance and supervision of Professor Dr. Most Ferdousi Begum, Department of Botany, University of Rajshahi and Dr. Shafiqul Islam, Assistant Scientist, Environment Microbiology Laboratory, ICDDRB, Dhaka . It is now submitted as a thesis for the Degree of Master of Philosophy (MPhil) in the Department of Botany, University of Rajshahi, Bangladesh and has not submitted for any other degree.

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It is our pleasure to certify that the research work presented in this dissertation entitled **"HYGIENE PRACTICE, MICROBIAL DIVERSITY AND DETECTION OF PATHOGENIC BACTERIA IN SOME READY-TO-EAT FOODS OF RAJSHAHI METROPOLIS"** is an original work done by Kanta Debnath for partial fulfillment of the requirements of the degree of Master of Philosophy in Botany. This thesis embodies the original work done by her and has not been submitted in substance of any other degree.

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Dedicated

To my

Beloved Parents

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The Author

ABSTRACT

RTE food has become an important public health issue and a great concern to everybody. This is due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety issues. In the present study different restaurants and street food vans of six hot spots of Rajshahi Metropolis were surveyed during March, 2015 to February, 2016. The survey was mainly conducted to ascertain the socioeconomic condition, hygiene and sanitation practice of food seller to reduce the contamination rate and to protect public health. From the survey result it was observed that all RTE food vendors of Rajshahi Metropolis were male with the age range as below 25-50 years, illiterate to under H.S.C pass and have 5-20 years of experience in this profession. The majority of restaurants vendors earned 26000 to 30000 taka monthly. The most of the restaurant vendors had no knowledge about food hygiene and very much irregular for cleaning of sale surfaces (23.07%). Hand washing before and after serving was practiced by 23.07% vendors, 30.77 % used aprons and none of vendors covered hair with caps or used gloves while serving food. They used detergent for cleaning of utensils. None of the vendors have any training about food safety and not aware of food safety law. On the other hand, in case of street food vendors, it was observed that majority (100%) of street vendors were male. Most of the vendors (60%) were 36-45 years old and 64% were passed JSC and only 8% street vendors were illiterate. The majority (52%) of street vendors earned 11000 to 15000 taka monthly and majority (80%) had no knowledge about food hygiene. None of the street vendors used apron, gloves while selling food and did not covered food with polythene. They have little knowledge about food hygiene but their food safety practices were poor.

Microbial quality of RTE foods of Rajshahi Metropolish was assessed. From the results it was observed that total viable count (TVC) of boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti contained $>10^6$ cfu/gm which is not safe for health. Large number of coliforms were recorded in different RTE food samples and hundred percent samples of boiled rice, vegetable curry,

chicken curry, fish, cooked dal and 25-75% of fuchka and chatpoti were contaminated with $>10^4$ cfu/gm. *E. coli* contamination were recorded as hundred percent for boiled rice, fuchka and chatpoti with $>10^2$ cfu/gm and not detected in cooked dal and fish curry at court areas. *Salmonella-Shigella* were not recorded in boiled rice and vegetable curry but 0-100% percent samples of chicken curry, cooked dal, fish curry, fuchka and chatpoti were contaminated with $>10^1$ cfu/gm. *Staphylococcus* was not recorded in vegetable curry, fish curry and chicken curry but 0 to 34% samples of boiled rice, 0 to 50% samples of vegetables curry and cooked dal; and 25 to 100% samples of fuchka and chatpoti contained $>10^6$ cfu/gm. Mean bacterial load of different RTE food items at different locations were analyzed. The maximum mean cfu of TVC (13.32 $\log_{10}$cfu/gm), coliform (10.94 $\log_{10}$cfu/gm), *E. coli* (13.12 $\log_{10}$cfu/gm), *Staphylococcus* (8.64 $\log_{10}$cfu/gm) and *Salmonella* (5.17 $\log_{10}$cfu/gm) was recorded for fuchka, fish curry, fuchka, fuchka and chicken curry, respectively. Moreover, the maximum number of TVC, *Staphylococcus,* coliforms, *E. coli* and *Salmonella* were recorded at Laxmipur and minimum at Shaheb Bazar.

Total 1140 isolates were isolated from the RTE foods. On the basis of colony morphology, Gram staining, motality and catalase test results these isolates were primarily grouped into 62. Then the isolates were selected for biochemical test according to Bergy's Manuals and confirmed by ABIS & Micro Rao online bacteria identification software. These isolates were identified as ten genera namely *Proteus, Edwardsiella, Klebsiella*, *Salmonella*, *Escherichia, Citrobacter*, *Enterbacter, Bacillus, Staphyloccous* and *Providencia.*

The frequency and abundance of the identified genera were determined. The maximum frequency of *Enterobacter* sp. (42.2%), *Citrobacter* sp. (53.3%), *Citrobacter* sp. (64.4%), *Proteus* sp. (64.4%), *Citrobacter* sp. (53.3%), *Staphylococcus* sp. (70%) and *Enterobacter* sp. (80%) were determined in boiled rice, cooked dal, vegetable curry, fish curry, chicken curry, fuchka and chatpoti, respectively. In overall, the most frequent genera was recorded as *Ctrorobacter* (46.6%) the lowest was *Bacillus* (6.14%). The maximum abundance of *Proteus* sp.

(28.71%), *Edwardsiell (7.9%), Klebsiella*, (8.51%), *Salmonella*(18.1%), *Escherichia (*14.28%), *Citrobacter*(33.87%), *Enterbacter(38.7%) Bacillus(12.5%) Staphyloccous(33.3%)* and *Providencia(11.65%) were recorded in fish curry, cooked dal, vegetable curry, cooked dal, fuchka, chatpoti, chatpoti,boiled rice, fuchka, chicken curry respectively.*

In overall, *Citrobacter* sp was the most prevalent (23.3%) genera followed by *Enterobacte*r (22.19%), *Salmonella* sp (9.47%), *Proteus* sp (8.7%), *Klebseilla* sp (5.78%), *Edwarseilla* sp (3.68%), *Proveidencia* sp(3.24%). and the least dominanting genera was *Bacillus* sp (3.07%). On the other hand, *Enterobacte*r sp, *Citrobacter* sp, *E. coli* and *Staphylococcus* sp. were common genera found in all types of RTE food items.

The rank of the bacteria for total abundance were as *Ctrobacter* > *Enterobacter* > *Staphylococcus*> *E.coli*> *Salmonella*> *Proteus*> *Klebseilla* > *Edwarsilla* > *Provedencia*> *Bacillus.*

Out of 62 isolates, nine isolates showed positive β-hemolytic activity. Among the nine isolates, SMRS-671, BAFM-417 and RSVE-185 no isolates showed highly toxic reactivity and the mice were died within 3 days and as well as for SMRM-651 and RKFE-227 no isolates mice died within 6 days and for rest of the isolates severe to mild illness symptoms apeared. Further, five tested isolates *Salmonella* sp. (SMRS-671) *Enterobacter* sp. (BAFM), *E. Coli* (RSVE-185) *Proveidencia* sp. (RKFE-227), *Proteus vulgaris* (SMRM-651) showed positive results for hem-agglutination.

From the results it is concluded that RTE food vendors of Rajshahi Metropolis have little knowledge about food hygiene but their food safety practices were poor. Moreover, RTE foods in this city were heavily contaminated by different microorganisms. These results also indicate that consumption of RTE foods may pose a risk of foodborne disease. So, it is suggested that regular monitoring of the quality of RTE foods must be practiced to avoid any food-borne pathogenic outbreaks in future and to ensure public health.

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ABBREVIATIONS

WHO	:	World Health Organization
NA	:	Nutrient Agar
EMB .	:	Eosine Methaline Blue
SS	:	*Salmonella-Shigella* Agar
MS	:	Manitol Salt.
TSI	:	Triple Sugar Iron
MR	:	Methyl Red
VP	:	Voges-Proskauer
mm	:	Millimeter
mg	:	Milligram
gm	:	Gram
mμ	:	Milimicron
μl	:	Microliter
mg/l	:	Milligram/liter
Min (s)	:	Minutes (s)
0.1N	:	0.1 Normal
No.	:	Number
pH	:	Negative logarithm of hydrogen
sp.	:	Species
etc.	:	et cetra, and the rest
Fig	:	Figure
et al.	:	et alli and the rest
i.e.	:	id est-which to say in other words
viz.	:	Namely
RTE	:	Ready-To-Eat food
FAO	:	Food and Agricultural Organization
ISO	:	International Organization for Standardization

HACCP	:	Hazard Analysis and Critical Control Points
CDC	:	Centers for Disease Control
USA	:	United States of America
^{0}C	:	Celsius
°F	:	Fahrenheit
a_w	:	Water Activity
cfu	:	Colony Forming Unit
U.S	:	United States
%	:	Percentage
RU	:	Rajshahi University
SSC	:	School Secondary Certificate
JSC	:	Junior School Certificate
ml	:	Mililitre
L	:	Litre
lb	:	Pounds
TVC	:	Total Viable Count
Gr	:	Group
R	:	Boiled rice
V	:	Vegetable curry
D	:	Cooked dal
F	:	Fish curry
C	:	Chicken curry
Fu	:	Fuchka
Ch	:	Chatpoti
ACP	:	Aerobic Plate counts
RTC	:	Ready-to-Cook foods
UP	:	Uttar Pradesh, India
AMC	:	Aerobic Mesophilic Count

Chapter One

Introduction

INTRODUCTION

Ready-to-eat foods are foods that will not be cooked before serving foods are intended to be consumed as they are. These foods do not require additional cooking and are usually stored in refrigeration or at room. Ready-to-eat (RTE) foods have become increasingly popular in the last two decades, particularly in metropolitan areas (Peck et al., 2008). It is being served quickly, testy and available at reasonable rates and attracts all the age groups, specially the young generations. Street foods are ready to eat foods and beverages prepared or sold by vendors and hawkers especially in streets and similar public places (FAO, 1989). Usually RTE food are not hygienic, to keep the attention of customer and earn more money with less effort and vendors compromise with food quality. People suffer a lot due to consumption of unhygienic foods.

RTE food items are being contaminated by the different microorganisms at alarming rate in our country as well as all over the world. Microorganisms produce pathogenic bacteria, viruses and toxins; it has ability to contaminate of all types of food. Food-borne pathogens are the leading causes of illness and death in developing countries and killing near about 1.8 million people annually. In developed countries food-borne pathogens are responsible for millions of cases of infectious gastrointestinal disease each and every year, costing billions of dollars in medical treatment. New food-borne pathogens and food-borne diseases are likely to emerge driven by factors such as pathogen evolution, changes in agricultural and food manufacturing practices, and changes to the human host status The World Health Organization defines food borne illnesses as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through

the ingestion of food (WHO, 2007).. Food borne diseases have a great impact on health; therefore, food safety is a worldwide major object (Velusamy et al., 2010).

Food borne bacterial pathogens commonly detected in RTE foods are *E. coli*, *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus* and *Salmonella* sp. (Vecdet Öz *et al.,* 2014). *E. coli* frequently contaminates food and a good indicator of fecal pollution (Benkerroum *et al.*, 2004). Presence of *E. coli* in RTE indicates the presence of entero pathogenic microorganisms, which constitute a public health hazard. Entero pathogenic *E. coli also* causes severe diarrhea, vomiting infants and young children. Certain foods sold by street vendors, e.g. vegetable, dal, fuchka, beef, fish and rice have been implicated in food poisoning outbreaks (Mensah *et al.,* 2012). The most commonly reported practice that contributed to food borne disease was bare-handed contact by handler/worker/preparer. Most consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality and hygiene. Under these circumstances, microbiological evaluation can often be very helpful to ascertain the safety and avoid public health hazards.

1.1 Ready-to-eat foods and health

Ready to eat food are an important source of nutrition with economic value mostly for the urban people. The health's of a significant number of urban dwellers are affected when food prepared in this way is contaminated due to the lack of a constant and insufficient heat treatment. One of the major contributors to bacterial contamination is the cooking of foods that are supposed to be served hot, but are made up in advance and stored for long periods of time before being served. When served, they are often not

reheated to a sufficient temperature to kill the harmful bacteria. The Food and Agricultural Organization (FAO, 1988) of the United Nations suggest that several cited food poisoning outbreaks in cities of the developing world, often leading to fatalities, could be traced back to the consumption of ready to eat foods.

In Nigeria, it was found that most of the ready to eat foods were prepared in bulk and displayed for a whole day for sale at ambient temperature without re-warming (Umoh and Odoba 1999). Foods at warm temperature for this period of time have risk and the risk increases substantially with every additional hour. It is a need to reduce the problems of ready to eat food contamination, growth of microorganisms and intoxication through education of ready to eat food sellers and the public on the importance of environmental sanitation and safe practices in the holding of cooked foods. Temperature is the most effective element for contamination of food by various microorganisms.

1.2 Food safety

Food safety is a scientific discipline describing handling, preparation, and storage of food in ways that prevent food borne illness. This includes a number of routines that should be followed to avoid potentially severe health hazards. In this way 'Food Safety' often overlaps with food defense to prevent harm to consumers. The tracks within this line of thought are safety between industry and the market and then between the market and the consumer. In considering industry to market practices, food safety considerations include the origins of food including the practices relating to food labeling, food hygiene, food additives and pesticide residues, as well as policies on biotechnology and food and guidelines for the management of governmental import and export inspection and

certification systems for foods. In considering market to consumer practices, the usual thought is that food ought to be safe in the market and the concern is safe delivery and preparation of the food for the consumer.

Food can transmit disease from person to person as well as serve as a growth medium for bacteria that can cause food poisoning. In developed countries there are intricate standards for food preparation, whereas in lesser developed countries the main issue is simply the availability of adequate safe water, which is usually a critical item (FAO, 1989). ISO 22000 is a standard developed by the International Organization for Standardization dealing with food safety. It specifies the requirements for a food safety management system that involves with interactive communication, system management, prerequisite programs, HACCP principles. A 2003 World Health Organization (WHO) report concluded that about 30% of reported food poisoning outbreaks in the WHO European Region occur in private homes. According to the WHO and CDC, in the USA alone, annually, there are 76 million cases of foodborne illness leading to 325,000 hospitalizations and 5,000 deaths (Bhowmik S.K., 2005). In 2003, the WHO and FAO published the Codex Alimentarius which serves as a guideline to food safety (Ghosh *et. al.*, 2007).

1.3 Microbiological risk factors

The most established microbiological problem facing the food industry is simple spoilage by bacteria, yeasts or molds that are not hazardous to health. The tiniest life forms are bacteria, yeasts, molds, and viruses, termed "microorganisms" because of their size.

Bacteria

Bacteria are the most important microorganisms to the food processor. Most are harmless, many are highly beneficial, some indicate the probable presence of filth, disease organisms, spoilage and a few cause diseases. There are thousands of species of bacteria, but all are single-celled and fall into three basic shapes: spherical, straight rods, and spiral rods. All bacteria reproduce by dividing into two cells. The two cells then divide to become 4, 4 become 8, and so forth. Under ideal conditions, this doubling may occur as frequently as every 15 minutes, so that within 5 hours there will be more than a million cells from the original single cell. If there are 1000 original cells instead of a single one, there will be over 1 billion cells in 5 hours. Some rod-shaped bacteria are capable of existing in two forms, dormant spores and active vegetative cells. Vegetative cells form spores under adverse conditions as a means of survival. Spore forms preserve the bacteria from starvation, drying, freezing, chemicals, and heat. When conditions become favorable, the spores germinate, with each spore again becoming a vegetative cell with the ability to reproduce. Among the bacteria, sporulation is not a means of reproduction since each cell forms a single spore which later germinates into a single cell again. One of the most studied food-borne pathogens is *Salmonella spp. Salmonella* species are enteric bacteria and can be found in the intestinal tract of animals, including birds. As such, *Salmonella* can frequently be isolated from raw foods of animal origin. Environmental contamination can also result in *Salmonella* being present in a wide variety of foods, although generally at lower numbers. Their presence in ready-to-eat foods may be a result of undercooking, poor handling practices and cross contamination. *Salmonella* can occasionally be isolated from fresh fruit and vegetables, and these may be a source of contamination when included in ready-to-eat

food. *E. coli* is an organism that is part of the normal micro flora of the intestinal tract of humans and warm-blooded animals. As such, their presence in ready-to-eat foods (fully cooked or those containing raw fruits or vegetables) can be an indication of poor hygiene and sanitation or inadequate heat treatment. *Bacillus cereus* can be detected in many raw foods of plant origin and in raw milk. As with *C. perfringens*, their spores will survive cooking, and poor temperature control after cooking may result in germination of the spores and subsequent growth. *B. cereus* is of greatest concern in plant or cereal based ready-to-eat foods and cream based sauces. Ready-to-eat foods containing raw components may contain low levels of *B. cereus*. *Staphylococcus aureus* can be routinely isolated from humans and associated environments. As such, the presence of coagulase positive *Staphylococci* (a subgroup of *S. aureus*), is an indication of human contact. Some CPS strains produce a toxin which can cause food poisoning. Even minimal handling of foods can result in coagulase positive *Staphylococci* being present in foods at low levels. This is unlikely to be a food safety concern provided the food is either consumed immediately or stored under temperature control. Extensive handling and/or temperature abuse may result in increased levels and increased food safety risk if toxin production occurs. In raw or fermented foods, coagulase positive *Staphylococci* are likely to be outgrown or inhibited by the naturally occurring microorganisms. In fully cooked foods that support the growth of coagulase-positive *Staphylococci*, the absence of competing microorganisms may provide the opportunity for growth and toxin production. Coagulase-positive *Staphylococci* appear to be of greatest concern in products such as custard or cream-filled pastries, mayonnaise-based salads and processed meats.

Yeasts and Molds

Yeasts are oval-shaped and slightly larger than bacteria. They reproduce most often by budding. In budding each cell can produce several buds, or swellings, which break away to form new, fully formed daughter cells. Molds as found on bread, fruit, damp paper, or other surfaces are actually composed of millions of microscopic cells joined together to form chains. The chains usually have numerous branches, called hyphae. Molds can thrive in conditions too adverse for bacteria or yeasts. They reproduce by spores that are frequently present as green or black masses on the protruding hyphae. Yeasts and molds grow on most foods, on equipment, and building surfaces where there are small amounts of nutrient and moisture. Since bacteria grow faster, they greatly outnumber yeasts and molds in most foods. However, bacteria find conditions of low pH, moisture, or temperature and high salt or sugar unfavorable. In such environments, yeasts or molds predominate. Thus, they can be a problem in dry foods, salted fish, bread, pickles, fruits, jams, jellies, and similar commodities.

Viruses

Viruses are the smallest and simplest microorganisms. Unlike bacteria, viruses, yeasts and molds are incapable of reproducing independently. Instead, they must first invade the cells of another living organism called the host, before they can multiply. Hence, they are parasitic. Viruses are normally specific in their selection of host cells, some infecting but one species, while others are capable of infecting closely related species. As a result, viruses which infect bacteria, called bacteriophages, cannot infect human beings or other animals. On the other hand, several animal viruses, known as zoonotis, can infect human beings. As disease transmitted by

food to human beings. Although viruses require a live host cell and cannot multiply in foods, they can remain viable and infectious for long periods of time, even under highly adverse conditions, such as drying, freezing, and pasteurization.

1.4. Sources of contamination of RTE foods

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 (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. 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; Muinde and Kuria, 2005; Ghosh *et al.*, 2007; Rane S, 2011). Insects and rodents carry pathogens from garbage, waste water 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). Each day, a few people coming to work in a shop or food preparation industry. They can potential spreaders of pathogenic microorganisms (eg. *Salmonella*). Most of the day laborer doesn't cut their fingertips properly and they are not conscious about personal hygiene. That's way they become permanent carriers of pathogens. Therefore, most of the local RTE food shop and their handling materials are unhygienic and contaminated. Coliform have probably got more attention than most other groups of bacteria on account of their importance as indicator organism for predicting unhygienic conditions during production and processing. *Klebsiella sp.* has been recognized as a food born pathogen (Kaclikova *et al.*, 2001) that can contaminate RTE foods (Menendez *et al.*, 2001). The following table is listed of some pathogens that commonly originate from human sources (Aneja *et al.*, 1990).

Table1.1. Contamination sources of RTE foods

Microorganisms	**Sources**
Shigella spp., *Hepatitis A, Norwalk virus, E. coli, Salmonella* spp., *Giardia lamblia*	Feces
Streptococcus group A	Throat and skin
Staphylococcus aureus	Skin, nose, boils

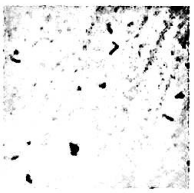
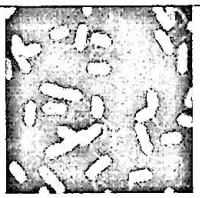
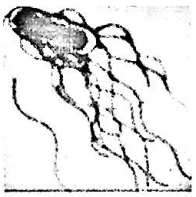
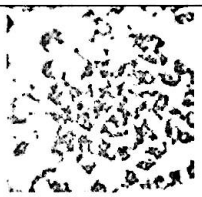
1.5 Necessary to study food borne pathogen

Food-borne illness is a major public health problem globally including Bangladesh. Recent U.S estimates indicate that some 76 million illnesses and attributed to food-borne disease 30% are caused by bacteria, 3% by parasites and 76% by viruses (Mead *et al.,* 1999). Microbiological agents, especially bacteria, are probably the most common cause of food borne diseases. Foods are the primary sources of many pathogenic microorganisms *Escherichia coli, Salmonella sp, Klebsiella oxytoca, Proteus mirabilis*, *Edwardsiella tarda* etc. These organisms commonly carried by the nose and the skin can easily be transferred to food through handling which causes infectious diseases like nausea, vomiting or fever Incidence of food-borne diseases are common in Bangladesh where public health and sanitation facilities are inadequate (Kabirullah, 2006) that's why various kinds of microorganisms cause food-borne diseases that are presented in Table 1.2.

Table 1.2. Major microorganisms associated with food-borne diseases

Pathogen	Microscopic image of pathogen	Typical incubation period (time between eating and onset of symptoms)	Typical symptoms	Typical food vehicles
Bacillus cereus toxin (vomiting and diarrhoea)		1 - 6 hours 6 - 24 hours	Sudden onset of severe nausea and vomiting Abdominal cramps, nausea and watery diarrhoea	Improperly refrigerated cooked rice and pasta, and fresh noodles Meats, stews, gravy

Table 1.2: Continue

Campylobacter		2 - 5 days	Fever, nausea, abdominal cramps and diarrhoea (sometimes bloody)	Raw and undercooked poultry, unpasteurised milk and contaminated water
Clostridium perfringens toxin		6 - 24 hours	Abdominal cramps, watery diarrhoea and nausea	Meats, poultry, gravy, dried or precooked foods
Escherichia coli including (STEC)		2 - 10 days more commonly 3 - 4 days	Diarrhoea (often bloody), abdominal cramps	Improperly cooked beef, unpasteurised milk and juice, sprouts and contaminated water
Listeria monocytogenes		3 days - 10 weeks	Meningitis, sepsis, fever	Soft cheeses, unpasteurised milk, ready-to-eat deli meats
Salmonella		6 – 72 hours, usually 12-36 hours	Headache, fever, abdominal cramps, diarrhoea, vomiting and nausea	Undercooked poultry, raw egg desserts and mayonnaise, sprouts, tahini
Staphylococcus aureus toxin		0.5 – 8 hours	Sudden onset of vomiting and abdominal cramps	Cream desserts and pastries, potato salad
Vibrio parahaemolyticus		4–30 hours, usually 12-24 hours	Nausea, vomiting, abdominal cramps and watery diarrhea	Undercooked or raw seafood.

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1.6 Potential bacteria present in RTE foods

To improve the microbiological quality of RTE foods precaution must be taken during food preparation because contamination may occur at different critical control points. Millions of people are the victims of food-borne illnesses resulting from toxins produced by *Staphylococcus aureus* ingested with food. The important pathogens have separated in various food groups into infective organisms (vegetative cells), which can be controlled by pasteurization, but toxins and spore-producing microorganisms are not controlled by pasteurization. The list of potential pathogen in food has given below:

Table 1.3. Potential bacteria present in RTE foods

Food	Pathogen
Boiled rice	*Bacillus cereus* *Salmonella spp.* *Escherichia coli* *Proteus spp.*
Vegetable curry	*Staphylococcus aureus* *Listeria monocytogenes* *E. coli* *Edwardsiella tarda* *Klebsiella oxytoca*
Chicken curry	*Staphylococcus aureus* *Salmonella spp.* *Shigella spp.* *Campylobacter jejuni* *Listeria monocytogenes*
Fish curry	*Staphylococcus aureus* *Salmonella spp.* *Shigella spp.* *Vibrio cholera* *Edwardsiella tarda* *Klebsiella oxytoca*

Source: Snyder *et al.* (1974) and internet

1.7 Factors affecting growth of microorganisms

The food processor reduces potential problems from microorganisms in several ways. Removing or destroying them by trimming, washing, heating, pickling, by adding chemicals or by encouraging competition by acid- or alcohol. Minimize contamination from equipment, people, and the environment and from unprocessed food. Minimize microbial growth on equipment by cleaning and sanitizing and in the product itself by adjusting storage temperature, pH, and other environmental factors. Although each factor affecting growth is considered separately in the following discussion, these factors occur simultaneously in nature. When more than one condition is somewhat adverse to microbial growth, their inhibitory effects are cumulative. pH, temperature and water activity are the major factor that influence the growth and reproduction of microorganism. Some microorganism love low and others high pH. According to preference of temperature microbes are psychophilic, mesophilic and thermophilic it depends on nature of microorganisms.

Temperature

Temperature is the most efficient means to control microbial growth. Based on their tolerance of broad temperature ranges, microorganisms are roughly classified as follows:

i. Psychrophies grow only at refrigeration temperatures.

ii. Psychrotrophs grow well at refrigeration temperatures but better at room temperature.

iii. Mesophiles grow best at or near human body temperature but grow well at room temperature.

iv. Thermophiles grow only at temperatures about as hot as the human hand can endure and usually not at all at or below body temperature.

To be more specific about these temperature limits of growth is to enter the controversy that has continued since the infancy of microbiology, for there are many species that grow in temperature ranges overlapping these. However, for food microbiology these conclusions are pertinent. Some psychrotrophic microorganisms grow very slowly in foods below freezing, but usually not below 19°F. There are a few reports of growth, usually of molds, at 14°F, but no reliable reports of growth below that temperature. This means that the standard storage temperature for frozen foods, O°F does not permit microbial growth. However, many microorganisms survive freezing (Elliott and, Michener 1964). Most psychrotrophs have difficulty growing above 90°F. Most food borne disease organisms are mesophiles. A good rule of thumb is to store perishable foods below 40°F or above 140°F. In the temperature range where both mesophilic and psychrotrophic organisms grow (about 41°F to about 90°F), the psychrotrophs grow more rapidly, causing spoilage and at the same time frequently interfering with the growth of food borne disease organisms (Elliott and Michener, 1965).

Water Activity

Water activity (a_w) is a term describing the availability of water to microorganisms. It is only roughly related to percent moisture. Pure water has an a_w of 1.00, and the atmosphere above the water in a closed container will have an equilibrium relative humidity (ERH) of 100%.

Table 1.4 The water activity (a_w) limits for growth of principal foodborne disease organisms.

Microorganism	Minimal a_w for growth	Reference
Salmonella	0.945	Christian & Scott, 1953
Clostridium botulinum	0.95	Scott, 1957
Clostridium perfringens	0.93	Kang, et al., 1969
Staphylococcus aureus	0.86	Scott, 1962
Vibrio parahaemolyticus	0.94	Beuchat, 1974

Most bacteria fail to grow in a food or other medium where the a_w is lower than 0.94. Bacteria require a higher a_w than yeasts, which in turn require a higher a_w than molds. Thus, any condition that lowers the a_w first inhibits bacteria, then yeasts and finally molds (Elliott and Michener, 1965). But each species has its limits which are interrelated with other growth factors. Table 1.4 gives the a_w limits for growth of principal food borne disease organisms held under otherwise optimal conditions. Certain molds and bacteria can grow on fish immersed in saturated salt solution where the a_w is about 0.75. Some molds can grow in foods with a_w 0.62 – 0.65 (Elliott and Michener, 1965). At these lower limits growth is very slow. The a_w of fully dried foods, such as crackers or sugar is about 0.10 and such products are microbiologically stable because of this factor alone. The stability of intermediate moisture foods (aw 0.75 – 0.90), such as dried fruits, jams and soft moist pet foods depends on combinations of factors, such as low a_w, low pH, pasteurization, chemical additives and impervious packaging.

pH

pH has a profound effect on the growth of microorganisms. Most bacteria grow best at about pH 7 and grow poorly or not at all below pH 4. Yeasts

and molds therefore, predominate in low pH foods where bacteria cannot compete. The lactic acid bacteria are exceptions; they can grow in high acid foods and actually produce acid to give us sour milk, pickles, fermented meats and similar products. Some strains called Leuconostoc contribute off-flavors to orange juice. The pH values of certain foods are given in Table 1.5

Table 1.5 Mean pH values of selected foods (Lopez, 1987)

pH Value	Selected Foods
2.3	Lemon juice (2.3), Cranberry sauce (2.3)
3.0	Rhubarb(3.1),Applesauce(3.4), Cherries, RSP(3.4) Berries(3.0–3.9),Sauerkraut(3.5)Peaches(3.7),Orange-juice(3.7) Apricots (3.8)
4.0	Cabbage, red(4.2),Pears(4.2), Tomatoes (4.3)
4.6	Ravioli(4.6), Pimientos (4.7), Tomato-sauce(4.9)
5.0	Figs(5.0),Onions(5.2) Carroes (5.2) Green Beans (5.3), Beans with pork (5.3)Asparagus (5.5), Potatoes (5.5), Lima-beans(5.9),Tuna(5.9),Tamales(5.9)
6.0	Codfish(6.0),Sardines(6.0),Beef(6.0), Pork(6.1),Evaporated-milk(6.1), Frankfurters(6.2),Chicken(6.2), Corn(6.3), Salmon (6.4), Crabmeat(6.8),Milk(6.8), Ripe-olives(6.9)
7.0	Hominy (7.0)

The lowest pH limits for growth of food borne disease organisms are shown in Table 1.6. Many of the investigators who reported these values also determined that adverse factors, such as low temperature or low water

activity, increased the minimal pH for growth. But the processor can be sure that these minimal values will prevent growth of these pathogens under any and all circumstances.

Table 1.6. The minimal pH for growth of principal foodborne disease organisms

Microorganism	Growth reported at but not below pH	Reference
Staphylococcus aureus	4.5	
Salmonella, Clostridium botulinum	4.0	Chung and Goepfer, 1970
Types A and B	4.8	National Canners Assn., 1971a
Type E	5.0	National Canners Assn., 1971a
Clostridium perfringens	5.0	
Vibrio parahaemolyticus	4.8	Beuchat, 1973
Bacillus cereus	4.9	Kim and Goepfer, 1971

1.8 Food borne illness hazards, threshold and quality levels

Food borne illness in commonly called food poisoning even though most cases are caused by a variety of pathogenic bacteria, viruses, prisons or parasites that that cause food contamination. Food-borne illness usually arises from improper handling, preparation or food storage. Spores of the pathogenic bacteria *B. cereus, C. botulinum,* and *C. perfringens* must germinate after the food is cooked, and the vegetative cells must multiply to a level of at least 10^4 to 10^6 cfu in order to make the food a hazard to consumers. *Campylobacter jejuni* is a principal cross-contamination

problem. The levels of microorganisms that causes illness, based on volunteer feeding tests of healthy people. Hazardous levels of foreign objects in food are also listed in (Table-1.3). Restaurant food borne out break were also reported in many countries, large out breaks in 2005, 2011 reported by different media (Medscope).

Table 1.7 Food-borne illness hazard, threshold and quality levels

Bacteria	Name of microorganisms	Healthy person (estimated illness dose)	Suggested purchaser food quality criteria
Spore forming	*Bacillus cereus*	10^5 to10^{11} CFU	<10^2 CFU/g
	Clostridium botulinum	3x10^3 CFU	<10 CFU/g
	C. perfringens	10^6 to10^{11}CFU	<10^2 CFU/g
Vegetative bacteria	*Escherchina coli*	10^6 to>10^{10}CFU	<10 CFU/g
	E. coli 0157:H7	10 to 100 CFU	<1 CFU/g
	Salmonella spp.	1to10^9 CFU	<1 CFU/g
	Shigella flexneri	10^2 to>10^9 CFU	<1 CFU/g
	Staphylococcus aureus	10^5 to>10^9 CFU	<10^2 CFU/g
	Vibrio cholerae	10^3 CFU	<1 CFU/g
	Yersinia enterocolitica	3.9×10^9 CFU	<10^2 CFU/g
	Listeria monocytogenes	> 10^3 to>10^5 CFU	<10 CFU/g

1.9 Global Impact of food borne illness and death

Food-borne illness is a common phenomenon around the world like United States, France, Australia and including Asia. 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). Even in industrialized countries up to 30% of the populations of people have been reported to suffer from food borne diseases every year. In the U.S around 76 million cases of food borne diseases, which resulted in 325,

00 hospitalizations and 5,00 deaths are estimated to occur each year (Centers for Disease Control, 2008). Centers for Disease Control and Prevention report estimate 1999 cases of food borne illness and deaths for the general population in U.S (Table 1.8). Restaurant food born out break was also reported in many countries large outbreak in 2005, 2011 reported by different media (Medscape).

Table 1.8. Food-related illness and death in United States

Agent	Name of Microorganism	ILLNESSES				
		Total cases estimated	Food borne	% of food borne transmission	%of food borne total	Death
Spore former	*Bacillus cereus*	27,360	27,360	100	0.2	2
	Clostridium perfringens	248,520	245,520	100	1.8	7
	C. botulinum	58	58	100	0.0	4
Vegetative bacteria	*E. coli 0157:H7*	78,480	1,963,141	80	14.2	99
	Listeria monocytogens	2518	2493	99	0.0	499
	Salmonella typhi	824	659	80	0.0	3
	Shigella spp.	488,240	89,648	20	0.6	14
	Staphylococcus spp.	185,060	185,060	100	1.3	2
	Streptococcus spp.	50,920	50,920	100	0.4	0
	Vibrio uvlnificus	94	47	50	0.0	18
	Yersina enterocolitica	96,368	86,731	90	0.6	2
	Grand total	1,173,442	2,654,637			650

Source: Mead *et al.* (1999)

1.10 Scererio of RTE food in Bangladesh

In Bangladesh street food vending is distinctive and prevailing part of large informal sector. Different items like snacks such as cake, biscuit, nuts, shingara, 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 and small size. This is the easiest way to income money in rapid urbanization of Dhaka city (Muzaffar, *et al.,* 2009) and about 200000 street food vendors are working in Dhaka city, as many workers choose the street vending as profession. 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 (Mensah , *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 standard restaurants. Microbial load of ready-to-eat rice in standard fast food centers is higher than the local fast food centers. On the other hand, Rajshahi is one of the largest city in Bangladesh and lives 0.77 million people. There are so many educational institutes, market and shopping places. Many of the street vendors are working in this city and their best choice as vending sites as besides these institutes, shopping mall, rail station, bus stand, road side etc. There is no rules and regulation and legal authorities to monitor microbial quality of

RTE foods sold in the city. Moreover A large number of students and passersby regularly eat RTE foods that contribute to health threat.

However, there is no report on microbial quality of RTE foods in Rajshahi city. In this circumstance the present investigation has been under taken to survey on sanitary quality of RTE foods and vending site of Rajshahi city; and microbial quality and microbial diversity of these foods were also analyzed.

Chapter Two

Review of Literature

REVIEW OF LITERATURE

Ready-to-eat (RTE) foods are available in the market, road side which are easily contaminated with different pathogenic bacteria and create a high risk of food poisoning. Thus more hygienic preventive measures are required to reduce the bacterial contamination to increase the wholesomeness of these RTE foods. In this regard, some researchers have studied microbial quality of food and antibacterial activity of spices against several bacteria. A brief review about such efforts are given below

Mosupye *et al.* (1999) reported that 51 ready-to-eat street foods, 18 dishwater, and 18 surface swab samples were collected from six vendors in Johannesburg, South Africa. Standard methods were used to determine aerobic plate counts (APCs), spore counts (SCs), and *Enteroabacteriaceae* counts (ECs) for food samples as well as coliform counts (CCs) for water and swab samples. Mean APC values of 3.4 (±0.4) log CFU/g, 4.0 (±1.2) log CFU/ml, and 2.1 (±0.4) log CFU/25 cm^2 were obtained for food, water, and swab samples, respectively. Mean SC values of 1.6 (±0.2) log CFU/g and 1.5 (±0.3) log CFU/25 cm^2 were obtained for food and swab samples, respectively. A mean EC value of 2.0 (±0.4) log CFU/g for food samples and mean CC values of 2.5 (±0.3) log CFU/ml and 1.3 (±0.3) log CFU/25 cm^2 for water and swab samples, respectively, were determined. Mean PC values of 1.6 (±0.1) log CFU/g, 1.9 (±0.6) log CFU/ml, and 1.4 (±0.4) log CFU/25 cm^2 were determined for food, water, and swab samples, respectively. *Bacillus cereus* was detected in 22%, *Clostridium perfringens* in 16%, *Salmonella* spp. in 2%, and *E. coli* (non-O157:H+) in 2% of the 51 food samples

Sharit and Bhowmik (2005) assessed the magnitude of street vending indifferent countries and the composition of the vendors. Most of South Asian developing cities have a large number of street vendors as an informal trade in the main urban transaction points as well as Dhaka city.

Martins and com (2006) surveyed street food venders in Gautang, South Africa and reported that most of them maintained high standard hygiene during food preparation and serving corroborated by the microbial count results.

Hosein *et al.* (2008) determined the prevalence and microbial load of *Listeria* spp., *Escherichia coli* O157 and *Salmonella spp.* in ready-to-eat products in supermarkets across Trinidad. The microbial load was assessed using the total aerobic plate count (TAPC) per g/ml of foods and prevalence of *Escherichia coli* O157 and *Salmonella spp.* determined using conventional methods. For *Listeria monocytogenes*, immunomagnetic separation (IMS), TECRA (enzymelinked immunosorbent assay, ELISA) and conventional methods were used. The log10 mean ± sd TAPC per g or ml was highest for vegetables (11.0±11.6), and lowest for seafood (5.2±5.7) ($p < 0.05$). The prevalence of *L. monocytogenes* was 1.7 %. Sixteen (4.5%) of 153 samples yielded *E. coli* but all samples were negative for *Salmonella spp.* and *E. coli* O157. The high microbial load and isolation of *L. monocytogenes* and *E. coli* from popular RTE foods could pose a health risk to consumers in the country.

Tambekar *et al.* (2008) analysed street vended foods in several places of India. The bacterial pathogens identified were *P. aeruginosa* (39%), *E.coli* (21%), *S. aureus* (16%), *Salmonella sp.* (12%) and *Proteus sp.* (12%). The highest frequencies of occurrence of bacterial pathogens were *P.*

aeruginosa in samosa (25%), *E. coli* in kachori (32%), *S. aureus* in kachori (27%), *Proteus sp.* in palakwada (45%) and 36% *Salmonella* sp. in samosa.

Meldrum *et al.* (2009) a survey of the general microbiological quality of ready-to-eat food served in schools was undertaken across Wales, United Kingdom. Of the 2,351 samples taken, four were identified as containing unsatisfactory counts of *Escherichia coli*, four contained unsatisfactory counts of *Staphylococcus aureus*, and one contained an unacceptable count of *Bacillus cereus* when compared with guidelines for the microbiological quality of ready-to-eat food published by the United Kingdom Public Health Laboratory Service in 2000. No samples contained detectable levels of *Salmonella, Listeria species*, or *Clostridium perfringens.* When compared with data on the general microbiological quality of food available in Wales, the food sampled from schools was of relatively better microbiological quality.

Abdalla *et al.* (2009) evaluated the food safety knowledge and practices of street food vendors in Atbara city between March and April, 2008. The questionnaires respondents were 28% male and 72% were female, 48% of them had primary school education while 42% were illiterates. The most prevalent isolated bacteria from cooked meals, bottled drink and fresh juice were *Escherichia coli, Staphylococcus auerus* and *Bacillus* sp. The viable bacterial counts were 4.6 CFU/ml, 3.7 CFU/ml and 4.1 CFU/ml for cooked meals, bottled drink and juice, respectively.

Das *et al.* (2010) studied the microbiological quality of street foods like panipuri and bhelpuri sold in many parts of Bangalore, India. Eight different samples of these chaats were aseptically collected from four locations of Bangalore City. The samples were analyzed within an hour of

procurement. Isolation, enumeration and identification of the prevalent bacteria and fungi were carried out following the standard procedures. Analysis of the food samples revealed high loads of bacterial pathogens such as *Streptococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus sp.*, *Klebsiella sp.* and *Pseudomonas* sp. Total viable counts of bacteria in all the samples varied between $0.4\text{-}3.0\times10^4$ cfu g^{-1}, faecal coliforms between $0.03\text{-}0.14\times10^4$ cfu g^{-1} and faecal streptococci between $0.2\text{-}11\times10^4$ cfu g^{-1}. Aciduric yeasts like *Saccharomyces* and filamentous fungi like *Mucor* and *Rhizopus* were also encountered. *Salmonella* and *Vibrio cholerae* were not detected in any of the samples.

Almualla *et al.* (2010) studied microbiological safety and shelf life of some RTE salads sold in supermarkets in Sharjah Emirate, United Arab Emirates. Samples of four RTE salads, tabbouleh, hummus, Greek salad, and coleslaw, were obtained from supermarkets and examined for aerobic bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*. The results indicated significant differences ($P\pm0.05$) for the aerobic bacteria count among the RTE salad types. In tabbouleh, hummus, Greek salad, and coleslaw, *E. coli* counts of 0.9, 0.50, 0.27, and 0.25 log most probable number (MPN)/g and total aerobic bacterial plate counts of 3.57, 2.71, 2.76, and 2.52 $\log_{10}$ cfu/g, respectively, were found after preparation (day 0). At day 0, all RTE salads tested except tabbouleh contained an acceptable count of total bacteria, but the aerobic bacteria count for all the RTE salads increased rapidly during storage from day 2 to day 6, regardless of the storage temperature (5, 25, and 40°C). Twenty percent of all samples contained *E. coli*, although the numbers were as low as 1 log MPN/g. No *S. aureus*, *L. monocytogenes* or *Salmonella* sp. serotypes were detected in any of the RTE salads tested in this study.

Chung *et al.* (2010) investigated the levels of total aerobic plate counts (APC), coliforms and various pathogens in convenient foods including ready-to-eat foods (RTE), ready-to-cook foods (RTC) and fresh-cut produce (FCP) in Korea were investigated. A total of 244 samples comprising 145 samples of RTE, 39 samples of RTC and 60 samples of FCP were purchased from hyper chain stores located in six different provinces. APC levels of RTE showed a relatively wide range of 1.9–7.8 $\log_{10}$cfu/g, while APC levels of RTC and FCP were relatively high at 6.3–8.1 $\log_{10}$cfu/g. Coliform contamination was detected in approximately 50% of the samples at levels up to 5 $\log_{10}$cfu/g. *Escherichia coli* was most frequently detected in convenient foods, and followed by *S. aureus*. *Salmonella spp.* and *Listeria monocytogenes* were detected in only one sample and *Vibrio parahaemolyticus* was not detected in any sample.

Iroha *et al.* (2011) reported that *C. jejuni, L. monocytogenes, Salmonella, S. aureus* and Verotoxic *E. coli* have been recognized as most frequent cause of food poisoning. In the United States, incidence of food borne illness is documented through Food Net. The bacterial agents responsible for most cases of food borne illness are *Salmonella, Campylobacter* and *Shigella* from ten pathogens tracked by Food Net. The estimated number of cases and mortality rate are considered for bacterial, viral and parasitic cases of food borne illness, *Salmonella* causes 31% of food related deaths, followed by *Listeria* (28%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 (3%).

Rane (2011) reported that street food vending has become an important public health issue and a great concern to everybody. This is due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety

issues. Various studies have identified the sources of food safety issues involved in street foods to be microorganism belonging to the genus *Bacillus*, *Staphylococcus*, *Clostridium*, *Vibrio*, *Campylobacter*, *Listeria*, *Salmonella.* Application of sound risk analysis policies is being advocated to provide a scientific base to the host of risk management option which India may need to explore to ensure public health and safety.

Cho *et al.* (2011) evaluated the microbial quality of street-vended foods in Korea. The microbiological contamination levels of aerobic mesophilic bacteria ranged from 0 to cfu/g, while the log1.53±cfu/g with a mean value of 4.71log, 7.43 cfu/g with a mean log level of total coliforms ranged from 0 to 6.86 cfu/g. log1.40±value of 3.24 *Staphylococcus aureus* (SA) and *Escherichia coli* cfu/g) and 3% log0.56±(EC) were detected in 9% (mean value: 3.75 cfu/g) of 326 examined samples, log0.90±mean value: 2.33 respectively. *Vibrio parahaemolyticus, Salmonella spp.* and *Listeria monocytogenes* were not detected.

Okolie *et al.* (2012) investigated a total of sixty-four samples of four cooked foods from sixteen private primary schools in Akoka area of Yaba, Lagos, Nigeria were investigated for the occurrence of pathogenic bacteria. The area was divided into four zones, namely; Akoka North, Akoka South, Akoka East and Akoka West using the four cardinal points. Four private primary schools were then selected from each of these zones. The standard plate count revealed that for Akoka North, the summary of the average microbial load for the rice, beans, meat and spaghetti samples are 3.5×10^4 cfu/ml, 6.9×10^4 cfu/ml, 4.9×10^4 cfu/ml, and 2.4×10^4 cfu/ml respectively; while the microbial load for similar sample in Akoka south are 2.9×10^4 cfu/ml, 6.8×10^4 cfu/ml, 4.9×10^4 cfu/ml and 2.4×10^4 cfu/ml respectively; whereas in Akoka East it is 3.3×10^4 cfu/ml, 4.6×10^4 cfu/ml, 4.6×10^4

cfu/ml and 2.5×10^4 cfu/ml; and in Akoka West, it is 2.9×10^4 cfu/ml, 4.7×10^4 cfu/ml, 4.9×10^4 cfu/ml and 2.2×10^4 cfu/ml. Further examination of the isolates revealed the presence of rod and cocci shaped bacteria. Some of these isolates were identified as *E. coli, S. aureus, Pseudomonas spp.* and *B. cereus* these are possible pathogens.

Yasin *et al.* (2012) studied to ascertain the food quality for human consumption and the bacteriological analysis was carried out in a prospective study in collaboration with National Institute of Health, Islamabad. The surveillance work was conducted during the months of July to August peak summer season to evaluate the objective parameters for assessment of the bacteriological and hygienic status of food being consumed in the peri-urban area of Islamabad and Rawalpindi. Out of a total of 91 samples, the various bacterial contaminants such as *E. coli, S. aureus, Salmonella* and fungal species were isolated and identified using standard cultural and biochemical methods. It was observed that in total food samples, 25.5% were positive for *E. coli*, 16.6% for *S. aureus* and 11.1% for *Salmonella* species.

Sandra *et al.* (2012) reported that *Bacillus cereus* (*B. cereus*) isolates are toxigenic and can cause food poisoning. Cooked rice is a potentially hazardous food, especially in tropical countries. The aim of this study was determined the prevalence of *B. cereus* and *B. thuringiensis* in raw and cooked rice marketed in Selangor, Malaysia. A combination of Most Probable Number - Polymerase Chain Reaction (MPN-PCR) method was used to detect gyrB gene in *B. cereus* and *B. thuringiensis*. Five local varieties of raw rice samples were negative for *B. thuringiensis* but all (100%) were positive for *B. cereus*. A total of 115 cooked rice samples (nasi lemak, nasi briyani, nasi ayam and nasi putih) were studied for the

presence of *B. cereus* and *B. thuringiensis*. Nasi ayam was found to have the highest prevalence (100%) of *B. cereus* compared to nasi putih (76.2%) and nasi lemak (70.4%). Nasi briyani had the lowest prevalence (50%) of *B. cereus*. The frequencies of *B. thuringiensis* were found to be 10, 30 and 35.2 % in nasi putih and nasi ayam, nasi briyani and nasi lemak respectively. Occurrence of *B. cereus* and *B. thuringiensis* in the samples ranged from < 3 to 1100 MPN/g in different samples. Maximum number of *B. cereus* was observed in nasi lemak, nasi briyani and nasi putih (> 1100 MPN/g) while nasi ayam showed less contamination (460 MPN/g) with *B. cereus* which was significantly different ($P < 0.05$) from others. The number of *B. thuringiensis* in nasi lemak, nasi briyani, nasi putih and nasi ayam were found to be >1100, 93, 9.2 and 3.6 MPN/g, respectively.

Nyenje *et al.* (2012) assessed the microbiological quality of various ready-to-eat foods sold in Alice, South Africa. Microbiological analysis was conducted on 252 samples which included vegetables, potatoes, rice, pies, beef and chicken stew. The isolates were identified using biochemical tests and the API 20E, API 20NE and API Listeria kits; results were analyzed using the one-way-ANOVA test. Bacterial growth was present in all the food types tested; high levels of total aerobic count were observed in vegetables, 6.8 ± 0.07 followed by rice, 6.7 ± 1.7 while pies had the lowest count (2.58 ± 0.24). Organisms isolated included: *Listeria spp.* (22%), *Enterobacter spp.* (18%), *Aeromonas hydrophila* (12%), *Klebsiella oxytoca* (8%), *Proteus mirabilis* (6.3%), *Staphylococcus aureus* (3.2%) and *Pseudomonas luteola* (2.4%). Interestingly, *Salmonella spp.* and *Escherichia coli* were not isolated in any of the samples. There was a statistically significant difference ($p < 0.05$) in the prevalence of foodborne pathogens from hygienic and unhygienic cafeterias.

Oranusi *et al.* (2013) investigated the microbial quality of snacks (ready to eat foods) sold in Ota, Ogun State was investigated. A total of 100 different samples from 3 vending sites namely, a University Cafeteria, a top class snacks bar and a local kiosk were analyzed for total aerobic plate count, coliform count and for specific pathogens and fungi. The University Cafeteria had mean total aerobic plate count and coliform count ranging from 1.1×103-3.0×104and 1.0×102-2.2×103.The snacks bar had mean total aerobic plate count and coliform countranging from 2.0×103-5.8×105 and 1.4×102-1.8×105 while the local kioskhad mean total aerobic plate count and coliform count ranging from 2.1×03-5.4×105and 1.0×102-8.0×104, respectively. The fungal counts from the three sites are within 1.0×102-4.0×102. Six different bacterial and three fungal isolates were identified to include *E. coli, S. aureus, Bacillus cereus, Enterococcus, Klebsiella* spp., *Pseudomonas* spp., *Aspergillus niger, Penicillium* spp. and Mucor.

Adjrah *et al.* (2013) studied microbiological quality of ready-to-eat (RTE) vegetable salads served by food shops in Lomé. The data were gathered from 45 food shops owners using semi structured questionnaires. The results showed that the street food sector is dominated by females, and 71.11% of them attended school. They lacked training on food hygienic and sanitary practice, and personal hygiene was not observed. *Salmonella spp.* was not detected in any of the samples evaluated, but almost 25% of the samples were contaminated by *S. aureus*. The percentage of samples positive for indicator of food safety lack germs like Total aerobic bacteria, Total coliforms, Thermo tolerant coliforms were 100, 100 and 37.68 respectively; corresponding to conformity rates of 14.49, 11.59 and 81.16 respectively. As for Aerobic sulfite reducing bacteria, yeast and mould, the

percentages were respectively 11.59, 78.26 and 72.46 with conformity: 92.75, 68.11 and 94.20. The level of the microbial contamination of the RTE salads collected from collective eating places in Lomé may present a potential health hazard to consumer.

Mamun *et al.* (2013) assessesd the microbiological quality of local food items vended by the school-based street food vendors in Dhaka City. A total of 80 schools from 19 school-zones of Dhaka City and its outskirts were chosen for the study. A total of 110 food samples, one each from 110 school-based street food vendors, were collected for laboratory analysis. Microbiological criteria recommended by the International Commission on Microbiological Specifications for Foods (ICMSF) were considered to classify food samples as 'satisfactory' (total coliforms <100 per g or ml) and 'unsatisfactory' (total coliforms ≥100 per g or ml). Of the 110 school-based street food samples analyzed in the laboratory, 44% were unsatisfactory. Among different items of street vended foods, 54% of sliced fruits samples, 59% of jhalmuri samples, 29% of chotpotis samples, 53% of vajavuji samples, and all (100%) sharbat samples were unsatisfactory, while all samples from achar (100%) and ice cream (100%) were found to be satisfactory.

Gawande *et al.* (2013) aimed to correlate socioeconomic profile of street food vendors and the microbiological quality of Samosa and Panipuri served by food vendors in Allahabad City, (U.P.) India. The data were gathered from 21 food vendors using semi structured questionnaires.The results showed that the street food sector is dominated by males, and 28.5% of them attended primary school and only 9% were graduate. 33.3% vendors washed their hands before and after the preparation of food. 76% vending site were found to be dirty but 57% vendors showed

clean personal hygiene. 80% water samples were showed presence of coliforms. The microbiological result of food item showed the presence of *Salmonella* (75%) in Samosa and 60% in Panipuri, Yeast and Mold (36%) in Samosa and (81%) in Panipuri.

Elobeid *et al.* (2014) studied microbiological quality of 14 different Qatari foods sold in Souk Wagif, the main traditional market in Qatar. A total of 56 samples were purchased from 10 different street vendors and the samples were collected randomly on different days. The qualitative study was conducted using Real Time-PCR to screen for; *Salmonella spp.*, *Listeria monocytogenes, Escherichia coli* and *E. coli* 0157:H7. Out of the 56 samples, only two samples "Biryani" and "Khabess" contained E. coli. However both samples tested negative for *E. coli* O157:H7. The microbial contamination of the Qatari traditional street vended foods was 3%.

Khairuzzaman *et al.* (2014) reported the street foods play an important socioeconomic role in meeting food and nutritional requirements of city consumers at affordable prices to the lower and middle income people

Öz *et al.* (2014) studied the microbiological quality of ready-to-eat (RTE) foods produced at catering companies in Istanbul, Turkey. RTE food was collected from May 2009 to May 2010 in Istanbul and a total of 750 samples including 13 food types were examined for the presence of coliform bacteria, *E. coli, S. aureus, B. cereus, Salmonella spp.* and *L. monocytogenes*. Comparison with the Microbiological Turkish Food Codex (TFC) shows that 3.6% of 532 meats, 18.3% of 120 salads and 50% of 12 pastries were at an unacceptable level of microbiological quality. nHowever, all pasta (32 samples), puddings (41 samples) and patties (14 samples) were at an acceptable level of microbiological quality according

to the TFC. In the samples that were examined coliform bacteria, *E. coli, B. cereus* and *S. aureus* respectively were isolated to 4.8%, 2.4%, 2.25% and 6.4%. *Salmonella* spp. and *L. monocytogenes* were not detected.

Nurudeen *et al.* (2014) conducted this study to examine the general hygiene and sanitary practices of street food venders in Nigeria and 110 samples of street food venders were selected to represent 18% of street food venders in the study area. Data was collected using pre-test structured questionnaire and observation checklists. The relationships in the factors studied were determined. Food venders lacked basic training on hygiene and only 2.7% had formal training on food preparation. 63.6% acquired skills from parents while 33.7% acquired skills by self practice. 44.5% of vendors used their mouths to blow air into polythene bags to open, before using it to package foods for customers. 60.0% of the vendors prepared foods in unkempt environment with flies around the foods.

Mashak *et al.* (2015) investigated the recontamination of ready-to-eat (RTE) products during post-processing may be the cause of outbreaks of food-borne disease. In this study, a total of 150 RTE samples were obtained for bacteriological examination like coliforms, *E. coli*, *S. aureus*, *Salmonella*, *B. cereus*, *Psychrotrophic* bacteria and *Psychrophilic* bacteria. Various types of RTE food products that contained frozen (cooked and semi-cooked) and refrigerated (cooked) poultry meat foods, were purchased randomly periodically in January and March, 2012. 65% of cooked samples and 62% of semi cooked samples contain more than 10^2 CFU/g coliform, while *S. aureus* was more than 10^2 CFU/g in 35 and 40% of samples, respectively. Also 28% of cooked samples and 44% of semi cooked samples contained *E. coli*. 14% of all samples were contaminated by *Salmonella*. The results for enumeration of *B. cereus*, psychrophilic and

psychrotrophic microorganisms were 2/96 ± 0/09 log_{10} cfu /g, 5/02 ± 1/77 log_{10} cfu/g and 3/05±0/04 log_{10} cfu/g, respectively.

Fowoyo and Ali (2015) studied microbial quality four food samples namely fried chicken, fried rice, meat pie and cake from four different fast food restaurants in Lokoja. Ten bacterial species were identified as *Streptococcus sp.*, *Bacillus sp.*, *Staphylococcus sp.*, *Flavobacterium sp.*, *Proteus sp.*, *Escherichia coli*, *Klebsiella sp.*, *Salmonella sp.* and *Pseudomonas aeruginosa*. Four fungal species namely *Penicillium sp.*, *Rhizopus stolonifer*, *Mucor sp.* and *Aspergillus flavus* were isolated and identified in the samples. The bacterial count of food samples ranged from 7.0×10^3 to 2.23×10^5 cfu/g while coliform count ranged from 6.0×10^5 to 1.175×10^5 cfu/g and the total fungal count ranged from 1.4×10^3 to 5.75×10^4 cfu/g.

Coniglio *et al.* (2016) investigated the microbiological quality of marketable RTE salads was discussed in terms of Public Health concerns. A total of 100 samples of RTE mixed salads were collected from local supermarkets in Catania (Italy). The RTE packages were immediately transported to the laboratory and analyzed according to the international standard methods (ISO) for aerobic mesophilic count (AMC at 30°C) for *E. coli*, coliforms, *Salmonella* and *L. monocytogenes*. All the samples (100%) were positive for AMC (median concentrations 10^6-10^7 cfu/g), 25.00% were positive for coliforms (median concentrations 10^4-10^5 cfu/g) and 10.00% for *E. coli* (median concentrations $<10^2$ cfu/g). No sample was positive for *Salmonella* and *L. monocytogenes*.

Luminita *et al.* (2016) investigated the presence of five pathogens in food brought into the European Union by Moldavian citizens as personal goods and illegally sold in Romania in the vicinity of the border. The occurrence of *S. aureus* and *L. monocytogenes* was 7.5% and 8%, while *Campylobacter* spp., *E. coli* O157:H7, and *Salmonella spp.* were absent in all samples. *L. monocytogenes* sequence type 2, 9, 121, and 155, highly prevalent among foodstuffs worldwide, was also present among isolates from ready-to-eat food illegally sold in Romania, even at the same date of sampling, indicating cross-contamination during food handling. *S. aureus*. a types t449, t304, and t524 were most often isolated from raw-milk cheeses contaminated with 10^3–10^5 cfu/g, evidencing a contamination at herd level or unhygienic conditions during processing. *S. aureus* t011 and t3625, both included in the livestock-associated CC398, were isolated from pork lard and poultry meat.

Shafizi *et al.* (2016) determined of the presence of *S. aureus* in ready-to - eat (RTE) food, food premise sanitation level and the relationship between *S. aureus* presence in RTE food and the sanitation levels of food premises in the locality of the study. A total of 106 samples of RTE food were analyzed by MPN-PCR and the results confirmed that 56 (53%) samples contained *S. aureus*. Surveyed by type, cooked RTE food had 50 (45%) of the sample having *S. aureus* presence compared to raw RTE with only 56 (55%). As for risk assessment, a checklist containing nine (9) main parameters consisting of 40 variables as a basis, was conducted on 53 food premises to determine their sanitation levels. The results revealed that 49 premises (92%) are sanitary while the remaining 4 (8%) were categorized as unsanitary. In terms of risk level, 4 premises (8%) were categorized as 'high risk', 26 (49%) 'moderate risk', and the remaining 23 (43%) are at

'low risk'. The study found no significant relationship between the presence of *S. aureus* in RTE food with the level of sanitation of premises in Putrajaya.

Bereda *et al.* (2016) collected one hundred thirty-two samples of street foods from four 'kebeles' of Jigjiga City. Both descriptive and analytical statistical methods were applied. The majority of the street food vendors were women, 120(90.9%), with the average age group of 23–49 years(42.85%), and 99(66.7%) them were illiterate. The study revealed that 95(72%) of the food samples had pathogenic bacterial contaminations. Three different bacterial species were isolated: *E. coli* 68(51.5%), *S. aureus* 85(64.4%) and 26(19.7%) *Salmonella* species. The highest incidence of *S. aureus* 23/33(69%) was seen in 'Sambusa'; the highest incidence of *E. coli* 24/33(73.5%) was observed in 'Pasta', while the highest Salmonella incidence was observed in 'Ades'

Yunita *et al.* (2016) studied to thirty five samples from twenty two food sellers in eight elementary schools in two different time periods. The analysis results showed that none of the samples was contaminated by *L. monocytogenes, Salmonella spp* or *Vibrio spp*. Two samples (otak-otak and siomai) were contaminated by *S. aureu*. The contamination source tracing showed that the hands, equipment, and environment around the selling place were contaminated by *S. aureus*. The likelihood of illness by *S. aureus* after consuming food and snack for school children was 0.00103 or one case for every 972 servings.

Mafune *et al.* (2016) investigated the microbial safety of 28 food samples obtained from four different locations in Thohoyandou. Standard microbiological methods were used for enumeration of *Staphylococcus*

aureus and *Coliforms*. Results of the study show that isolated *S. aureus* was <2.4771 log10 cfu/g in all samples and locations, while there was no significant difference ($p>0.05$) in total coliform count (<2.1761 log10 cfu/g) among samples and across all locations. Microbial contamination of SVFs using the total plate count method was observed in the food samples except fried potato chips, with marked significant difference ($p<0.05$) recorded across locations in cereals and meat samples. Majority of SVFs obtained from all locations met the microbiological standard of ready-to-eat foods.

Osakue *et al.* (2016) studied fried chicken samples from two vending sites (from street vendors and fast food centers) in Ikpoba Okha, Oredo and Egor LG as in Benin City. The results obtained indicated that the mean values of bacteria count ranged from 4.67×10^6 to 1.2×10^7 cfu/g. The sample from the eatery in Oredo had the highest bacterial count of 1.2×10^7 cfu/g. Thirteen genera of bacteria and seven genera of fungi were isolated. Total fungal counts ranged from 4.70×10^5 to 2.615×10^6 cfu/g. The fried chicken from the street in Ikpoba Okha recorded the highest count with 2.615×10^6 cfu/g. The fried chicken samples from the eateries had no *E. coli* and other serious food-borne pathogens; such as *Klebsiella sp*, *Enterobacter sp.* The aerobic colony counts of bacteria and fungi were higher than International Food Standards ($<10^5$ cfu/g), not withstand the absence of food-borne microbial pathogens.

Isara *et al.* (2017) assessed the food hygiene and safety practices of mobile food vendors in Benin City, Nigeria. This descriptive cross-sectional study was carried out among mobile food vendors in Benin City, Nigeria. A structured, pretested, interviewer-administered questionnaire and an observational checklist were the data collection tools. Two hundred and

fifty respondents with a mean age of 31.7 ± 8.7 years were studied. There were 24 (9.6%) males and 226 (90.4%) females. Most of them knew regular hand washing practices with soap and water (95.5%), proper covering of stored food (92.8%), proper cleaning of cutting/sale surfaces (89.6%), and storage of food at appropriate temperature (79.6%). Forty-seven (18.8%) respondents kept long finger nails and 58 (23.2%) blew air into the cellophane. Hand washing before and after serving food was practiced by 115 (46.0%) respondents, 14 (5.6%) used hand gloves while serving food, 143 (57.3%) properly covered their hair, and 205 (82.0%) concurrently served food and collected money from consumers. The utensils used in serving food were considered clean in majority 217 (86.8%) of the food vendors.

Henry *et al.* (2017) studied the microbiological quality of six selected cooked ready-to-eat street foods (Moimoi, Afang Soup, Stew, Porridge Yam, Porridge Beans, and Jollof Rice) sold freely and openly. A total of five hundred and forty (540) cooked ready-to-eat street food samples were collected from three types of food vendors. Results obtained showed that the aerobic plate count, *Staphylococcus aureus* count, and *Escherichia coli* count for cooked ready-to-eat street foods sold at stationary vendors with shade (SVWS) ranged from 1.20×10^6 to 4.20×10^6 cfu/g for aerobic plate count, 1.20×10^6 to 3.6×10^6 cfu/g for *Staphylococcus aureus* count, and 0.9×10^6 to 2.50×10^6 cfu/g for *Escherichia coli* count respectively. For stationary vendors without shade (SVWOS), it ranged from 1.29×10^6 to 3.50×10^6 cfu/g for aerobic plate count, 1.60×10^6 to 4.00×10^6 cfu/g for *Staphylococcus aureus* count, 1.00×10^6 to 2.80×10^6 cfu/g for *Escherichia coli* count. Aerobic plate count, *Staphylococcus aureus* count, and *Escherichia coli* count for mobile vendors (MV) ranged from 1.80×10^6 to

5.00×10^6 cfu/g for aerobic plate count, 1.60×10^6 to 5.80×10^6 cfu/g for *Staphylococcus aureus* count, 1.20×10^6 to 4.20×10^6 cfu/g for *Escherichia coli* count, respectively.

Kassa and Azene (2017) assessed the microbiological quality and safety of street vended meat sauce in Bahir Dar Town. A total of 60 samples (30 each in morning and afternoon) were assessed to reveal indicator bacteria and pathogens from December, 2010 to June, 2011 using standard bacteriological techniques. Mean aerobic mesophilic count of $4.16\times10^4$and 4.71×10^4cfu/g were recorded in the morning and afternoon, whereas mean total coli form count and *S. aureus* were $4.00\times10^4$and $4.53\times10^4$and $3.52\times10^4$and 4.39×10^4cfu/g in the morning and afternoon, respectively. Only the count of aerobic mesophilic bacteria was below the recommended limit. The mean microbial loads were significantly different in the morning and afternoon ($p<0.005$).

Dahiya *et al.* (2018) investigated multiple fast food samples for the assessment of microbial contamination. Spring Rolls and Chowmein were reported to have highest bacterial load ranging from 105 to 106 cfu/ml amongest all the samples studied. Samples like Bhel-Puri and Pani-Puri, had relatively low bacterial counts in the range of 102 to 104 cfu/ml. Biochemical tests further confirmed the presence of *Bacillus sp.* and *Klebsiella sp.* in Spring Roll sample while *Escherichia coli* in Chowmein sample. Bhel Puri and Pani Puri samples had the predominance of *Salmonella sp.* However, the Burger, and the Samosa samples were found to be contaminated with *Pseudomonas sp.* and *Enterobacter sp.* respectively. Besides, coliform bacteria reported to be present in Pani-Puri sample

Sanlier *et al.* (2018) determined the preferences for RTE foods; a questionnaire was given to 847 individuals constituted by randomly selected high school and university students. Of the participants, 43.4% were male and 56.6% were female; the majority of them were between 19 and 22 years of age. It was found that 40.1% of the young people ate street food 2-3 times per week, whereas 23.3% were found to eat it every day. Turkish bagels, döner, boiled corn in a cup and toast are most preferred street foods. A statistically significant negative correlation was found between consumption preference scores and education, gender, and age.

Tonelada *et al.* (2018) aimed to investigate the food safety knowledge and practices of street food vendors and to assess the sanitary conditions of street vended foods of Tarlac State University, Main Campus. From the total of 30 respondents, majority of those involved in the food vending business are the female (60%). Half of the total of the respondents had attained secondary education (50%). Majority of the respondents (80%) equally did not have any training on food safety and were not aware of food safety law. Most of the respondents vended food items in an environment that is observed to be clean (3.57), with the presence of waste bin, hand towel, and hand soap. Vended foods are protected through covered food containers ensuring food safety. It was found from the results of the study that street foods were vended under relatively good sanitary conditions.

Chapter Three

Materials and Methods

MATERIALS AND METHODS

Survey: In the present study different restaurants and street food vans and vendors of six hot spots of Rajshahi Metropolis were surveyed during March 2015 to February 2016. The survey was conducted on the basis of some visual observations, structural questionnaires and interview.

3.1 Assessment of microbial quality of RTE food

3.1.1 Samples collection

Total seven types of RTE food items were collected from six hotspots namely Laxmipur, Court, Naodapara, Sahebazer, Binodpur, RU Campus of Rajshahi Metropolis. Among them, boiled rice, vegetable curry, cooked dal, fish curry, chicken curry were restaurant foods and fuchka and chatpoti were street foods. The samples were put into sterilized polybags and tied well with rubber band. Then the samples were carried out to the Plant Pathology, Mycology and Microbiology Laboratory immediately and stored at 4^0 C for further experiment.

3.1.2 Labeling of the samples

After collection the samples were labeled according to their sources (Table 3.1).

3.1.3 Serial dilution of the samples

Serial dilution technique was followed by Reiner (1982) method. At first 100 ml sterilized distil water was taken in a tube. Then 1gm of food sample was taken into the tube and shacked vigorously by vortex motor to make a homogenous suspension. This gave a dilution of $1:10^2$. Then 1ml of first dilution was transferred by a micropipette to another test tube, which containing 9 ml of sterilized distilled water to make the dilution of

1:10^3 and this way made upto10^6. This technique was applied for each sample.

3.1.4 Media used for detection of bacterial load of ready-to-eat (RTE) food samples

Nutrient agar MacConkey agar, EMB agar, SS agar and MS agar of media were used for enumeration of cfu of bacteria from RTE food samples that are presented below:

Nutrient agar (NA) medium

Nutrient agar is used for the cultivation of bacteria and for the enumeration of microorganism of dairy products, various foods and other materials. To prepare nutrient agar medium Downers and Ito (2001) method was followed with the following composition:

Components	gm/L
Beef Extract	3.0
Peptone	5.0
NaCl	5.0
Agar	15.0

Preparation: Beef extract 3.0 gm, peptone 5.0 gm, NaCl 5gm, Agar 15.0 gm were dissolved in 1 L of distilled water and mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder and autoclaved at 121°C for 15 minutes. After autoclave liquefy agar was cool to 45-50°C and pour into Petri dishes. Then it was solidifying for at least 30 minutes. When the medium become solid the Petri dishes were prepared for culture and stored at 4°C

MacConkey agar (MA) medium

MacConkey agar is a selective and differential culture medium designed to isolate and differentiate gram-negative and lactose fermenter. Bile salts and crystal violet inhibit the growth of gram positive organisms. Bile salts may also precipitate out of the media surrounding the growth of fermenters because of the change in pH. Non-fermenters will produce normally-colored or colorless colonies. MacConkey agar was prepared using following composition (Downers and Ito, 2001).

Components	gm/L
Peptone	17.0
Protease peptone	15
Sodium chloride	5.0
Lactose	10.0
Bile salt No. 3	15
Neutral red	0.03
Crystal violet	1.0
Agar	13.5

Preparation: At first 50.1gm MacConkey agar was taken in a beaker containing 1000 ml of distilled water and mixed well. Prepared medium was disposed into conical flasks (500 ml) through separating funnel. Finally, the culture flasks containing medium were autoclaved at 15 lb/inch^2 pressure and 121°C temperature for 20 min to ensure sterilization. For preparing of culture plates, sterilized petridishes and medium was taken under running laminar air flow as soon as possible. The laminar air flow was started for 30 minutes before the cul[illegible] process. The medium was cooled to 50-55°C. Then th[illegible]int of Pet[illegible]

was flamed slightly and then the cover was also flamed slightly with spirit lamp and then the sterilized medium was poured in equal amount (15 ml/plate). Then the petridishes (with medium) were allowed to cool and solidified the medium.

Eosin Methylene Blue (EMB) agar

EMB agar is a very versatile solid medium. Originally developed by Levine for the differentiation of *Escherichia coli* and *Aerobacter aerogenes*, it turned out to be effective for the rapid identification of *Candida albicans* and was found useful for the identification of coagulase-positive *Staphylococci* (Health protection agency, 2004)

Components	**gm/L**
Peptone	10.0
Lactose	10.0
Dipotassium monohydrogen phosphate	2.0
Methylene Blue	0.065
Eosine Y	0.4
Agar	15.0

Final pH was adjusted to 7.1 $\pm$ 0.2 at 25°C

Preparation: Peptone 10.0gm, lactose 10.0gm, dipotassium monohydrogen phosphate 2.0gm, methylene blue 0.065gm, Eosin Y 0.4gm and agar 15.0gm were dissolved in 1 L of distilled water and mix thoroughly. Then heat it with frequent agitation and boiled for 1 minute to completely dissolve the powder and autoclaved a[illegible]1°C fo[illegible] minutes. After autoclaved liquefy agar was cool to 45-[illegible]ur in[illegible] Petri dishes. Then it was solidifying for at least 30 mi[illegible]he

medium become solid the Petridis were prepared for culture and stored at below 8°C.

***Salmonella-Shigella* SS) agar**

SS agar (*Salmonella-Shigella* Agar) is a differential selective media used for the isolation of *Salmonella* sp. and some *Shigella* sp. from specimens, foodstuffs etc.

Components	**gm/L**
Beef extract	5.0
Peptic digest of animal tissue	5.0
Lactose	10.0
Bile salts mixture	8.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	15.0

Final pH 7.0±0.2 at 25°C

Preparation: Firstly 63.02 gm SS agar in 1000 ml distilled water and boiled with frequent agitation to dissolve the medium completely. Overheating may destroy selectivity of the medium, so overheating or autoclaving were not done. Cool it about 50°Cand mix into sterile Petri plates.

Mannitol Salt (MS) Agar Medium

Manitol Salt Agar is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus* from clinical and

non clinical specimens. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. It is a selective medium prepared according to the recommendation of Chapman for the isolation presumptive pathogenic *Staphylococci* sp.

Staphylococcus spp. ferment lactose produce yellow colonies in medium after 37°C for 48 hours incubation.

Components	**gm/L**
Sodium Chloride	75.0
Proteose Peptone	10.0
Mannitol	10.0
Beef Extract	1.0
Phenol Red	0.025
Agar	15.0

Final pH was adjusted to 7.1 ± 0.2 at 25°C

Preparation: Sodium Chloride 75.0gm, Proteose Peptone 10.0gm, Mannitol 10.0gm, Beef Extract 1.0gm, Phenol Red 0.025gm and agar 15.0gm were dissolved in 1 L of distilled water and mix thoroughly. Then heat it with frequent agitation and boiled for 1 minute to completely dissolve the powder and autoclaved at 121°C for 15-20 minutes. After autoclaved liquefy agar was cool to 45-50°C and pour into petridishes. Then it was solidifying for at least 30 minutes. When the medium become solid the Petridis were prepared for culture and stored at below 8°C.

3.1.5 Plating

Initially NA, MacConkey, EMB and SS, and MS agar medium were used for isolation of bacteria from RTE food samples. 0.5 ml of each diluted

sample was dropped by micropipette on the plates containing nutrient agar, EMB, SS and MacConkey, and MS agar then spreaded by glass spreader. The plates were sealed by Para film and incubated at 37°C for 24 hours. The growth of bacteria were screened through the morphological characteristics of the colonies.

3.1.6 Determination of colony forming unit (cfu)

After 24 to 48 hours of incubation, the plates with different colonies were selected for counting. Counting of colonies was normally done by visual observation. Then total cfu were determinate according to the following formula:

$$\text{cfu} = \frac{\text{Number of Colony}}{\text{Number of dilution (used to make plate for colony count)}} \times \text{Amount plated}$$

Discrete colonies were selected for isolation on the basis of their morphological characteristics. The selected colonies were marked and their characteristics were studied depending on the various points of view such as: elevation, margin, surface, color etc (Eklund and Lankford, 1967). Then the marked and observed colonies were transferred aseptically to fresh nutrient agar medium in Petridis.

3.2 Isolation, characterizations and identification of the bacterial isolates of RTE foods

3.2.1 Isolation and identification of the bacterial isolates

For isolation of bacteria from the RTE foods, NA, MacConkey, EMB, SS and MS agar medium were used and serial dilution technique was followed. The colonies on different medium were observed thoroughly and short out on their morphological variation.

3.2.2 Purification of the isolates

For pure culture preparation streaking method was followed. The inoculating loop was flamed to redness and cooled by touching on a medium surface. After this a marked single colony of each sample plate looped out from the agar plate and the edge fresh sterilized medium plate (NA) was streaked out with the inoculums from side to side in paralleled line or in zigzag pattern. The favorable conditions were maintained to prepare pure culture of bacteria.

3.2.3 Maintenance of the isolates

The purified bacterial isolates were maintained on sterilized nutrient broth (NB) agar during the course of investigation. The culture tubes tip was wrapped tightly by cotton plug. The isolates were transferred to fresh medium periodically.

3.2.4 Coding of the isolates

The pure culture of the isolates were coded according to the origin and type of sample used. These code numbers were maintained and followed till identification of the isolates through morphological, microscopically and biochemical characterizations. The codes of the isolates were presented in (Table 3.1-3.5).

Table: 3.1. Coding of isolates according to sources in Laxmipur

Food Item	NA	MacCky	EMB	SS	MS
Boiled Rice	LCRN-1	LCRM-11	LCRM-21	LCRS-31	LCRMn-913
	LCRN-2	LCRM-12	LCRM-22	LCRS-32	LCRMn-914
	LTRN-41	LTRM-51	LTRE-61	LTRS-71	LTRMn-923
	LTRN-42	LTRM-52	LTRE-62	LTRS-72	LTRMn-924
	LERN-81	LERM-91	LERE-101	LERS-111	LTEMn-933
	LERN-82	LERM-92	LERE-102	LERS-12	LTEMn-934
Cooked Dal	LCDN-9	LCDM-19	LCDE-29	LCDS-39	LCDMn-921
	LCDN-10	LCDM-20	LCDE-30	LCDS-40	LCDMn-922
	LTDN-49	LTDM-59	LTDE-69	LTDS-79	LTDMn-931
	LTDN-50	LTDM-60	LTDE-70	LTDS-80	LTDMn-932
	LEDN-89	LEDM-99	LEDE-109	LEDS-119	LEDMn-941
	LEDN-90	LEDM-100	LEDE-110	LEDS-120	LEDMn-942
Vegetable curry	LCVN-5	LCVM-15	LCVE-25	LCVS-35	LCVMn-917
	LCVN-6	LCVM-16	LCVE-26	LCVS-36	LCVMn-918
	LTVN-45	LTVM-55	LTVE-65	LTVS-75	LTVMn-927
	LTVN-46	LTVM-56	LTVE-66	LTVS-76	LTVMn-928
	LEVN-85	LEVM-95	LEVE-105	LEVS-115	LEVMn-937
	LEVN-86	LEVM-96	LEVE-106	LEVS-116	LEVMn-938
Fish curry	LCFN-7	LCFM-17	LCFE-27	LCFS-37	LCFMn-919
	LCFN-8	LCFM-18	LCFE-28	LCFS-38	LCFMn-920
	LTFN-47	LTFM-57	LTFE-67	LTFS-77	LTFMn-921
	LTFN-48	LTFM-58	LTFE-68	LTFS-88	LTFMn-922
	LEFN-87	LEFM-97	LEFE-107	LEFS-117	LEFMn-931
	LEFN-88	LEFM-98	LEFE-108	LEFS-118	LEFMn-932
Chicken curry	LCCN-3	LCCM-13	LCCE-23	LCCS-33	LCCMnn-915
	LCCN-4	LCCM-14	LCCE-24	LCCS-34	LCCMn-916
	LTCN-43	LTCM-53	LTCE-63	LTCS-73	LTCMn-925
	LTCN-44	LTCM-54	LTCE-64	LTCS-74	LTCMn-926
	LECN-83	LECM-93	LECE-103	LECS-113	LECMn-935
	LECN-84	LECM-94	LECE-104	LECS-114	LECMn-936
Fuckka	LFuNS-721	LFuMS-725	LFuES-729	LFuSS-733	LFuMnS-1093
	LFuNS-722	LFuMS-726	LFuES-730	LFuSS-734	LFuMnS-1.94
	LFuNS-723	LFuMS-727	LFuES-731	LFuSS-735	LFuMnS-1095
	LFuNS-724	LFuMS-728	LFuES-732	LFuSS-736	LFuMnS-1096
Chatpoti	LChNS-737	LChMS-741	LChES-745	LChSS-749	LChMnS-1117
	LChNS-738	LChMS-742	LChES-746	LChSS-750	LChMnS-1118
	LChNS-739	LChMS-743	LChES-747	LChSS-751	LChMnS-1119
	LChNS-740	LChMS-744	LChES-748	LChSS-752	LChMnS-1120

L=Laxmipur, C= Caferaj, T= Tripti, E= Eman Hotel, R= Rice, V= Vegetables curry, D= Cooked Dal, F= Fish curry, C= Chicken curry, F= Fuchka, C= Chatpoti, N= Nutrient Agar medium, M= MacConkey medium, EMB= Eosin Methylene Blue agar medium, SS= Salmonella Shigella agar medium, MS/Mn= Mannotol Salt agar medium,S=Street food.

Table 3.2. Coding of isolates according to sources in Court

Food Item	NA	MacCky	EMB	SS	MS
Boiled Rice	CMRN-241	CMRM-251	CMRM-261	CMRS-271	CMRMn-973
	CMRN-242	CMRM-252	CMRM-262	CMRS-272	CMRMn-974
	CKRN-281	CKRM-291	CKRE-301	CKRS-311	CKRMn-983
	CKRN-282	CKRM-292	CKRE-302	CKRS-312	CKRMn-984
	CBRN-321	CBRM-331	CBRE-341	CBRS-351	CBRMn-993
	CBRN-322	CBRM-332	CBRE-342	CBRS-352	CBRMn-994
Cooked Dal	CMDN-249	CMDM-259	CMDE-269	CMDS-279	CMDMn-981
	CMDN-250	CMDM-260	CMDE-270	CMDS-280	CMDMn-982
	CKDN-289	CKDM-299	CKDE-309	CKDS-319	CKDMn-991
	CKDN-290	CKDM-300	CKDE-310	CKDS-320	CKDMn-992
	CBDN-329	CBDM-339	CBDE-349	CBDS-359	CBDMn-1001
	CBDN-330	CBDM-340	CBDE-350	CBDS-360	CBDMn-1002
Vegetable curry	CMVN-245	CMVM-255	CMVE-265	CMVS-275	CMVMn-977
	CMVN-246	CMVM-156	CMVE-266	CMVS-276	CMVMn-978
	CKVN-285	CKVM-295	CKVE-305	CKVS-315	CKVMn-987
	CKVN-286	CKVM-296	CKVE-306	CKVS-316	CKVMn-988
	CBVN-325	CBVM-335	CBVE-345	CBVS-355	CBVMn-997
	CBVN-326	CBVM-336	CBVE-346	CBVS-356	CBVMn-998
Fish curry	CMFN-247	CMFM-257	CMFE-267	CMFS-277	CMFMn-979
	CMFN-248	CMFM-147	CMFE-268	CMFS-278	CMFMn-980
	CKFN-287	CKFM-297	CKFE-307	CKFS-317	CKFMn-989
	CKFN-288	CKFM-298	CKFE-308	CKFS-318	CKFMn-990
	CBFN-327	CBFM-337	CBFE-347	CBFS-357	CBFMn-999
	CBFN-328	CBFM-338	CBFE-348	CBFS-358	CBFMn-1000
Chicken curry	CMCN-243	CMCM-253	CMCE-263	CMCS-273	CMCMn-975
	CMCN-244	CMCM-254	CMCE-264	CMCS-274	CMCMn-976
	CKCN-283	CKCM-293	CKCE-303	CKCS-313	CKCMn-985
	CKCN-284	CKCM-294	CKCE-304	CKCS-314	CKCMn-986
	CBCN-323	CBCM-333	CBCE-343	CBCS-353	CBCMn-995
	CBCN-324	CBCM-334	CBCE-344	CBCS-354	CBCMn-996
Fuckka	CFuNS-753	CFuMS-757	CFuES-761	CFuSS-765	CFuMnS-1097
	CFuNS-754	CFuMS-758	CFuES-762	CFuSS-766	CFuMnS-1098
	CFuNS-755	CFuMS-759	CFuES-763	CFuSS-767	CFuMnS-1099
	CFuNS-756	CFuMS-760	CFuES-764	CFuSS-768	CFuMnS-1100
Chatpoti	CChNS-769	CChMS-773	CChES-777	CChSS-781	CChMnS-1121
	CChNS-770	CChMS-774	CChES-778	CChSS-782	CChMnS-1122
	CChNS-771	CChMS-775	CChES-779	CChSS-783	CChMnS-1123
	CChNS-772	CChMS-776	CChES-780	CChSS-784	CChMnS-1124

C=Court, M= Mouinuddin, K= Khabar Hotel, B= Bhai Bhai, R= Rice, V= Vegetables curry, D= Cooked Dal, F= Fish curry, C= Chicken curry, F= Fuchka, C= Chatpoti, N= Nutrient Agar medium, M= MacConkey medium, EMB= Eosin Methylene Blue agar medium, S= Salmonella Shigella agar medium, MS/Mn= Mannotol Salt agar medium,S=Street food.

Table 3.3. Coding of isolates according to sources in Nowdapara

Food Item	NA	MacCky	EMB	SS	MS
Boiled Rice	NTRN-481 NTRN-482 NMRN-541 NMRN-542 NERN-561 NERN-562	NTRM-491 NTRM-492 NMRM-551 NMRM-552 NERM-571 NERM-572	NTRM-501 NTRM-502 NMRE-561 NMRE-562 NERE-581 NERE-582	NTRS-511 NTRS-512 NMRS-571 NMRS-572 NERS-591 NERS-592	NTRMn-1003 NTRMn-1004 NMRMn-1013 NMRMn-1014 NERMn-1023 NERMn-1024
Cooked Dal	NTDN-489 NTDN-490 NMDN-529 NMDN-530 NEDN-569 NEDN-570	NTDM-499 NTDM-500 NMDM-539 NMDM-540 NEDM-579 NEDM-580	NTDE-509 NTDE-510 NMDE-559 NMDE-550 NEDE-589 NEDE-590	NTDS-519 NTDS-520 NMDS-559 NMDS-560 NEDS-599 NEDS-600	NTDMn-1011 NTDMn-1012 NMDMn-1021 NMDMn-1022 NEDMn-1031 NEDMn-1031
Vegetable curry	NTVN-485 NTVN-486 NMVN-525 NMVN-526 NEVN-565 NEVN-566	NTVM-495 NTVM-496 NMVM-535 NMVM-536 NEVM-575 NEVM-576	NTVE-503 NTVE-504 NMVE-545 NMVE-546 NEVE-585 NEVE-586	NTVS-515 NTVS-516 NMVS-555 NMVS-556 NEVS-595 NEVS-596	NTVMn-1007 NTVMn-1008 NMVMn-1017 NMVMn-1018 NEVMn-1027 NEVMn-1028
Fish curry	NTFN-487 NTFN-488 NMFN-527 NMFN-528 NEFN-567 NEFN-568	NTFM-497 NTFM-498 NMFM-537 NMFM-538 NEFM-577 NEFM-578	NTFE-507 NTFE-508 NMFE-547 NMFE-548 NEFE-587 NEFE-588	NTFS-517 NTFS-518 NMFS-557 NMFS-558 NEFS-597 NEFS-558	NTFMn-1009 NTFMn-1010 NMFMn-1019 NMFMn-1020 NEFMn-1029 NEFMn-1030
Chicken curry	NTCN-483 NTCN-484 NMCN-523 NMCN-524 NECN-563 NECN-564	NTCM-493 NTCM-495 NMCM-533 NMCM-534 NECM-573 NECM-574	NTCE-503 NTCE-504 NMCE-543 NMCE-544 NECE-583 NECE-584	NTCS-513 NTCS-514 NMCS-553 NMCS-554 NECS-593 NECS-594	NTCMn-1005 NTCMn-1006 NMCMn-1015 NMCMn-1016 NECMn-1025 NECMn-1026
Fuckka	NFuNS-881 NFuNS-882 NFuNS-883 NFuNS-884	NFuMS-884 NFuMS-885 NFuMS-886 NFuMS-887	NFuES-888 NFuES-889 NFuES-890 NFuES-891	NFuSS-892 NFuSS-893 NFuSS-894 NFuSS-895	NFuSMn-1101 NFuSMn-1102 NFuSMn-1103 NFuSMn-1104
Chatpoti	NChNS-896 NChNS-896 NChNS-897 NChNS-898	NChMS-899 NChMS-900 NChMS-901 NChMS-902	NChES-903 NChES-904 NChES-905 NChES-906	NChSS-907 NChSS-908 NChSS-909 NChSS-910	NChMnS-1125 NChMnS-1126 NChMnS-1127 NChMnS-1128

N=Nowdapara, T= Tusti, E= Easin, M= Maa Hotel, R= Rice, V= Vegetables curry, D= Cooked Dal, F= Fish curry, C= Chicken curry, F= Fuchka, C= Chatpoti, N= Nutrient Agar medium, M= MacConkey medium, EMB= Eosin Methylene Blue agar medium, S= Salmonella Shigella agar medium, MS/Mn= Mannotol Salt agar medium,S=Street food.

Table 3.4. Coding of isolates according to sources in Saheb Bazar

Food Item	NA	MacCky	EMB	SS	MS
Boiled Rice	SBRN-601 SBRN-602 SMRN-641 SMRN-642 SARN-681 SARN-682	SBRM-611 SBRM-612 SMRM-651 SMRM-662 SARM-691 SARM-692	SBRM-621 SBRM-622 SMRE-661 SMRE-662 SARE-701 SARE-702	SBRS-631 SBRS-632 SMRS-671 SMRS-672 SARS-711 SARS-712	SBRMn-1033 SBRMn-1034 SMRMn-1043 SMRMn-1044 SARMn-1053 SARMn-1054
Cooked Dal	SBDN-609 SBDN-610 SMDN-649 SMDN-650 SADN-689 SADN-690	SBDM-619 SBDM-620 SMDM-659 SMDM-660 SADM-699 SADM-700	SBDE-629 SBDE-630 SMDE-669 SMDE-670 SADE-709 SADE-710	SBDS-639 SBDS-640 SMDS-679 SMDS-680 SADS-719 SADS-720	SBDMn-1041 SBDMn-1042 SMDMn-1051 SMDMn-1052 SADMn-1061 SADMn-1062
Vegetable curry	SBVN-605 SBVN-606 SMVN-645 SMVN-646 SAVN-685 SAVN-686	SBVM-615 SBVM-616 SMVM-655 SMVM-656 SAVM-675 SAVM-676	SBVE-625 SBVE-626 SMVE-665 SMVE-666 SAVE-685 SAVE-686	SBVS-635 SBVS-636 SMVS-675 SMVS-676 SAVS-695 SAVS-696	SBVMn-1037 SBVMn-1038 SMVMn-1047 SMVMn-1048 SAVMn-1057 SAVMn-1058
Fish curry	SBFN-607 SBFN-608 SMFN-647 SMFN-648 SAFN-687 SAFN-688	SBFM-617 SBFM-618 SMFM-657 SMFM-658 SAFM-697 SAFM-698	SBFE-627 SBFE-628 SMFE-667 SMFE-668 SAFM-707 SAFM-708	SBFS-637 SBFS-638 SMFS-677 SMFS-678 SAFS-717 SAFS-718	SBFMn-1039 SBFMn-1040 SMFMn-1049 SMFMn-1050 SAFMn-1059 SAFMn-1060
Chicken curry	SBCN-603 SBCN-604 SMCN-643 SMCN-644 SACN-683 SACN-684	SBCM-613 SBCM-614 SMCM-653 SMCM-654 SACM-693 SACM-694	SBCE-623 SBCE-624 SMCE-663 SMCE-664 SACM-603 SACM-604	SBCS-633 SBCS-634 SMCS-673 SMCS-674 SACS-613 SACS-614	SBCMn-1035 SBCMn-1036 SMCMn-1045 SMCMn-1046 SACMn-1055 SACMn-1056
Fuckka	SFuNS-849 SFuNS-850 SFuNS-851 SFuNS-852	SFuMS-853 SFuMS-854 SFuMS-855 SFuMS-856	SFuES-857 SFuES-858 SFuES-859 SFuES-860	SFuSS-861 SFuSS-862 SFuSS-863 SFuSS-864	SFuSMn-1105 SFuSMn-1106 SFuSMn-1107 SFuSMn-1108
Chatpoti	SChNS-865 SChNS-866 SChNS-867 SChNS-868	SChMS-869 SChMS-870 SChMS-871 SChMS-872	SChES-873 SChES-874 SChES-875 SChES-876	SChSS-877 SChSS-878 SChSS-879 SChSS-880	SChMnS-1129 SChMnS-1130 SChMnS-1131 SChMnS-1132

S=Saheb bazar, B= Biddut, M= Memory, A= Ankur, R= Rice, V= Vegetables curry, D= Cooked Dal, F= Fish curry, C= Chicken curry, F= Fuchka, C= Chatpoti, N= Nutrient Agar medium, M= MacConkey medium, EMB= Eosin Methylene Blue agar medium, S= Salmonella Shigella agar medium, MS/Mn= Mannotol Salt agar medium,S=Street food.

Table 3.5. Coding of isolates according to sources in Binodpur

Food Item	NA	MacCky	EMB	SS	MS
Boiled Rice	BRRN-361 BRRN-362 BARN-401 BARN-402 BHRN-441 BHRN-442	BRRM-371 BRRM-372 BARM-411 BARM-412 BHRM-451 BHRM-452	BRRM-381 BRRM-382 BARE-421 BARE-422 BHRE-461 BHRE-462	BRRS-391 BRRS-392 BARS-431 BARS-432 BHRS-471 BHRS-472	BRRMn-1063 BRRMn-1064 BARMn-1073 BARMn-1074 BHRMn-1083 BHRMn-1084
Cooked Dal	BRDN-369 BRDN-370 BADN-409 BADN-410 BHDN-449 BHDN-450	BRDM-379 BRDM-380 BADM-419 BADM-420 BHDM-459 BHDM-460	BRDE-389 BRDE-390 BADE-429 BADE-430 BHDE-469 BHDE-470	BRDS-399 BRDS-400 BADS-439 BADS-440 BHDS-479 BHDS-480	BRDMn-1071 BRDMn-1072 BADMn-1081 BADMn-1082 BHDMn-1091 BHDMn-1092
Vegetable curry	BRVN-365 BRVN-366 BAVN-405 BAVN-406 BHVN-445 BHVN-446	BRVM-375 BRVM-376 BAVM-415 BAVM-416 BHVM-455 BHVM-456	BRVE-385 BRVE-386 BAVE-425 BAVE-426 BHVE-465 BHVE-466	BRVS-395 BRVS-396 BAVS-435 BAVS-436 BHVS-475 BHVS-476	BRVMn-1067 BRVMn-1068 BAVMn-1077 BAVMn-1078 BHVMn-1087 BHVMn-1088
Fish curry	BRFN-367 BRFN-368 BAFN-407 BAFN-408 BHFN-447 BHFN-448	BRFM-377 BRFM-378 BAFM-417 BAFM-418 BHFM-457 BHFM-458	BRFE-387 BRFE-388 BAFE-427 BAFE-428 BHFE-467 BHFE-468	BRFS-397 BRFS-398 BAFS-437 BAFS-438 BHFS-477 BHFS-478	BRFMn-1069 BRFMn-1070 BAFMn-1079 BAFMn-1080 BHFMn-1089 BHFMn-1090
Chicken curry	BRCN-363 BRCN-364 BACN-403 BACN-404 BHCN-443 BHCN-444	BRCM-373 BRCM-374 BACM-413 BACM-414 BHCM-453 BHCM-454	BRCE-383 BRCE-384 BACE-423 BACE-424 BHCE-463 BHCE-464	BRCS-393 BRCS-394 BACS-433 BACS-434 BHCS-473 BHCS-474	BRCMn-1065 BRCMn-1066 BACMn-1075 BACMn-1076 BHCMn-1085 BHCMn-1086
Fuckka	BFuNS-785 BFuNS-786 BFuNS-787 BFuNS-788	BFuMS-789 BFuMS-790 BFuMS-791 BFuMS-792	BFuES-793 BFuES-794 BFuES-795 BFuES-796	BFuSS-797 BFuSS-798 BFuSS-799 BFuSS-800	BFuMnS-1109 BFuMnS-1110 BFuMn-S1111 BFuMnS-1112
Chatpoti	BChNS-801 BChNS-802 BChNS-803 BChNS-804	BChMS-805 BChMS-806 BChMS-807 BChMS-808	BChES-809 BChES-810 BChES-811 BChES-812	BChSS-813 BChSS-814 BChSS-815 BChSS-816	BChMnS-1133 BChMnS-1134 BChMnS-1135 BChMnS-1136

B=Binodpur, H= Haji Biriani, R= Royel Hotel, A= Alamin, R= Rice, V= Vegetables cu[illegible] Dal, F= Fish curry, C= Chicken curry, F= Fuchka, C= Chatpoti. [illegible] Agar me[illegible] MacConkey medium, EMB= Eosin Methylene Blue agar mediu[illegible] lla Shige[illegible] medium, MS/Mn= Mannotol Salt agar medium,S=Street food

Table 3.6. Coding of isolates according to sources in RU Campus

Food Item	NA	MacCky	EMB	SS	MS
Boiled Rice	RCRN-121 RCRN-122 RSRN-161 RSRN-162 RKRN-201 RKRN-202	RCRM-131 RCRM-132 RSRM-171 RSRM-172 RKRM-211 RKRM-212	RCRM-141 RCRM-142 RSRE-181 RSRE-182 RKRE-221 RKRE-222	RCRS-151 RCRS-152 RSRS-191 RSRS-192s RKRS-231 RKRS-232	RCRMn-943 RCRMn-944 RSRMn-953 RSRMn-954 RKRMn-963 RKRMn-964
Cooked Dal	RCDN-129 RCDN-130 RSDN-169 RSDN-170 RKDN-209 RKDN-210	RCDM-139 RCDM-140 RSDM-179 RSDM-180 RKDM-219 RKDM-220	RCDE-149 RCDE-150 RSDE-189 RSDE-190 RKDE-229 RKDE-230	RCDS-159 RCDS-160 RSDS-199 RSDS-200 RKDS-239 RKDS-240	RCDMn-951 RCDMn-952s RSDMn-961 RSDMn-962 RKDMn-971 RKDMn-972
Vegetable curry	RCVN-125 RCVN-126 RSVN-165 RSVN-166 RKVN-205 RKVN-206	RCVM-135 RCVM-136 RSVM-175 RSVM-176 RKVM-215 RKVM-216	RCVE-145 RCVE-146 RSVE-185 RSVE-186 RKVE-225 RKVE-226	RCVS-155 RCVS-156 RSVS-195 RSVS-196 RKVS-235 RKVS-236	RCVMn-947 RCVMn-948 RSVMn-957 RSVMn-958 RKVMn-967 RKVMn-968
Fish curry	RCFN-127 RCFN-128 RSFN-167 RSFN-168 RKFN-207 RKFN-208	RCFM-137 RCFM-138 RSFM-177 RSFM-178 RKFM-217 RKFM-218	RCFE-147 RCFE-148 RSFE-187 RSFE-188 RKFE-227 RKFE-228	RCFS-157 RCFS-158 RSFS-197 RSFS-198 RKFS-237 RKFS-238	RCFMn-949 RCFMn-950 RSFMn-959 RSFMn-960 RKMnF-969 RKMnF-970
Chicken curry	RCCN-123 RCCN-124 RSCN-163 RSCN-164 RKCN-203 RKCN-204	RCCM-133 RCCM-134 RSCM-173 RSCM-174 RKCM-213 RKCM-214	RCCE-143 RCCE-144 RSCE-183 RSCE-184 RKCE-223 RKCE-224	RCCS-153 RCCS-154 RSCS-193 RSCS-194 RKCS-233 RKCS-234	RCCMn-949 RCCMn-950 RSCMn-959 RSCMn-960 RKCMn-969 RKCMn-970
Fuckka	RFuNS-817 RFuNS-818 RFuNS-819 RFuNS-820	RFuMS-821 RFuMS-822 RFuMS-823 RFuMS-824	RFuES-825 RFuES-826 RFuES-827 RFuES-828	RFuSS-829 RFuSS-830 RFuSS-831 RFuSS-832	RFuMnS-1113 RFuMnS-1114 RFuMnS-1115 RFuMnS-1116
Chatpoti	RChNS-833 RChNS-834 RChNS-835 RChNS-836	RChMS-837 RChMS-838 RChMS-839 RChMS-840	RChES-841 RChES-842 RChES-843 RChES-844	RChSS-845 RChSS-846 RChSS-847 RChSS-848	RChMnS-1137 RChMnS-1138 RChMnS-1139 RChMnS-1140

R=Rajshahi University, C= Cafeteria, S= Sagor canteen, K= Kalam Hotel, R= Rice, V= [illegible] curry, D= Cooked Dal, F= Fish curry, C= Chicken curry, F= Fuchka, C= [illegible]poti, N= N[illegible] medium, M= MacConkey medium, EMB= Eosin Methylene Blue a[illegible]um, S= Shigella agar medium, MS/Mn= Mannotol Salt agar medium,S=Street f[illegible]

3.2.5 Preservation of the isolates

The isolates were sub cultured on NA medium for further research. The following methods were followed for the preservation of the isolates

Stab culture

The strains grown in pure culture for 24 hours' incubation at 37°C were stabbed directly inside the 3 ml autoclaved media in separate small vials by sterile loop and incubated at 37°C for 24 hours with loose cap to grow bacteria. After incubation, the cap of the vials was sealed by para film and stored at room temperature.

Slant culture

Five ml of LB autoclaved medium was taken in separate sterile test tube. The test tubes with medium were kept at 30° angle for increasing the medium surface. Then from the earlier pure cultures, the bacterial strain was streaked in zigzag pattern in each test tube with LB medium and incubated at 37°C for 24 hours. After 24 hours of incubation, the bacterial strains formed colony. These cultured test tubes were stored at 4°C in refrigerator. It can be stored for 2-3 months. Occasionally sub-culturing was maintained to keep the culture in active conditions with unimpaired character.

Glycerol stock

At first sterilized the eppendrof tubes and tube stand by autoclave. Then 800µl, i.e. equal volume of the overnight incubated broth culture of the isolates collected from pure culture was added in each [illegible]ppendrof [illegible] separately. Then centrifuge the tubes and co[illegible] bacte[illegible] precipitation and added 400µl of 33% glycerol was a[illegible]h 2ml

eppendorf tubes. The cap of the eppendorf tubes were covered by Para film and stored at 20°C.

3.2.6 Identification and characterizations of the selected isolates

Identification and characterization of the microorganisms was a sequential process, which included a series of different types of experiments. In the present study the following sequence of experiments were carried out for the presumptive identification and characterization of the isolates:

3.2.6.1 Morphological or cultural characteristics of the bacterial isolate

The bacterial colonies grown on cultural plate containing different media (Nutrient agar, EMB, SS, MacConkey and MS agar) were studied as to their shape, edge, elevation, opacity etc. whether grown inside at the bottom or on the surface of the media in pour plate condition.

3.2.6.2 Microscopic observation

The sizes and shapes of vegetative cells were determined by microscope. The arrangement of the cells whether presents singly or in pairs were also observed. The following techniques were employed for microscopic examination of the isolates.

Gram staining

For gram staining Koby and Ronald's (1985) modified method was followed.

Gram staining was demonstrate using following reagents

- 1% crystal violet solution
- Iodine solution (1% iodine in 2% Kl)

- Ethanol (75%)
- 1% Safranine (counter stain)

Procedure: A sterile loop was used to transfer a loop full of tap water to a clean glass slide. A desired single bacterial colony was looped out and mixed in the water on the slide. The specimen was allowed to dry on the slide at room temperature. After drying, the specimen was heat-fixed on the slide. The slide was gently heated by passing quickly through the flame, specimen side up, 3-4 times. Then the fixed smear was treated with 1% crystal violet solution for 1 minute. After that the slide was gently washed in tap water for 2-3 seconds and 1-2 drops of iodine solution was added and kept it for another 1 minute. The slide was washed gently in tap water and shakes off excess water. After those, 3-4 drops of 95% ethanol were added on the slide and kept it for 10 seconds. The slide was then washed gently in tap water and shakes off excess water. Then 1-2 drops of 1% safranine (counter stain) solution were added for 30 seconds. Then the slide was washed with tap water and dried with blotting paper and finally observed under microscope.

3.2.6.3 Biochemical test of the selected isolates

The following biochemical test was carried out to characterize and identification of the isolated bacteria.

Motility test

This test is used to determine if an organism is motile or non-motile, because, to identify an unknown bacterium it is usually necessary to determine whether the organism is motile or not. For motility test h[illegible]ing drop method with slightly modifications method wa[illegible]lowed, [illegible] Protection Agency, 2007)

Composition of motility test medium

Component	Amount (gm/L)
Tryptose	10.0
Sodium Chloride	5.0
Agar	5.0

Procedure: Motility test medium was demonstrated the swimming ability of bacteria in a semisolid medium. A semisolid medium with 0.75% agar is inoculated with the bacteria in a straight-line stab with a needle. After incubation, if turbidity (cloudiness) due to bacterial growth can be observed away from the line of the stab, it was evidence that the bacteria were able to swim through the medium.

Indole test

Indole test was taken according to Holt *et al.,* (1994). This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. Indole is then tested by a calorimetric reaction with p-dimethyl-aminobenzaldehyde. For indole test tryptophan or peptone broth medium and Kovac's reagent were used.

Composition of peptone broth medium (pH: 7.0)

Component	Amount (gm/L)
Peptone (Containing sufficient tryptophan)	20
Sodium chloride	5
Distilled water	1000 ml

Media Preparation: All ingredients were di[illegible] in 1 [illegible] d water and mixed thoroughly and heated [illegible] The[illegible]

dispensed and autoclaved at 121°C for 20 minutes at 15lb/inch^{2}pressure.The pH the media was adjusted to 7.0.

Oxidase test

Oxidase test was carried out for the enzyme oxidase of unknown isolates. For oxidase test Goszczynska*et al.,* (2000) method was followed.

Composition of oxidase test medium

Components	Amount (gm/L)
N,N,N,N-tetramethyl-ρ-Phenylenediaminedihydrochloried	1
Distilled water	1000 ml

Media preparation: Media was prepared 1gm of N,N,N'N-p-phenylene diamine-dihydro-chloride dissolved in 100 ml of distilled water.

Procedure: For oxidase test wet filter paper method was followed. For this test a few drops of N,N,N,N–tetramethyl-ρ-phenylene diamine dihydro chlorid solution was placed on a new piece of Whitman No, 1 filter paper with a clean Pasteur Pipette. Remove part of a colony with a sterile loop. Then smear was done onto the moistened paper and color was changed to dark purple within 30 seconds was positive and it was takes longer up to 60 seconds, for widely positive.

Catalase test

Catalase test was performing to determine the ability of the organism to liberate gas (O_2) from hydrogen peroxide (H_2O_2) by enzymatic (catalase) reaction. Catalase test was carried out according to McFadden (1980).

Reagents: Hydrogen peroxide (H_2O_2).

Procedure: For this purpose, a small amount test organism was taken from agar plate or slant culture onto a slide. Then added a few drops of hydrogen peroxide to the smear. If bubbles become visible (these would be O_2 bubbling up) then the test is positive.

Citrate test

Simmons Citrate Agar is used for the differentiation of microorganisms on the basis of citrate utilization. Simmons Citrate Agar is recommended for differentiation of enteric Gram-negative bacilli from diarrheal samples.

Composition of Simmons citrate agar

Components	Amount (gm/L)
Ammonium Dihydrogen	1
Phosphate	1
Dipotassium Phosphate	5
Sodium Chloride	2
Sodium Citrate	0.2
Magnesium Sulfate	0.08
Bromthymol Blue	15
Agar	

pH: 6.9 ± 0.2 at 25°C

Principles of the procedure: Ammonium dihydrogen phosphate is the sole source of nitrogen in Simmons citrate agar. Dipotassium phosphate acts as a buffer. Sodium chloride maintains the osmotic balance of the medium. Sodium citrate is the sole source of carbon in this medium. Magnesium sulfate is a cofactor for a variety of metabolic reactions.

Bromthymol blue is the pH indicator. Organisms that can utilize ammonium dihydrogen phosphate and sodium citrate as their sole sources of nitrogen and carbon will grow on this medium and produce a color change from green (neutral) to blue (alkaline). Agar is the solidifying agent.

Procedure: 3ml of sterilized Koser's citrate medium was taken in a sterilized screw cap test tube and inoculate with fresh culture of test organism and incubate at 37°C for 4 days. Growth in medium observed by turbidity and a color change of the indicator from light green to blue (due to alkaline reaction) following citrate utilization indicated positive and no growth in.

Kovac's reagent

Component	Amount (ml/L)
Amyl or isomyl alcohol	150
p-dimethyl-aminobenzaldehyde	10
Concentrated HCl acid	50

p-dimethyl-aminobenzaldehyde was dissolved in amyl alcohol and then added the HCl acid slowly. This reagent was stored at 4^0C and was shaken gently before use.

Procedure: Tubes of tryptophan broth were inoculating with the test organism from agar culture plate and incubated for 48-72 hours at 37^0C. Sometimes a period of 96 hours was required for optimum accumulation of indol. A few drops of Kovac's reagent were added to the broth culture. A red color in the alcohol layer was the indication of positive reaction.

Methyl Red (MR) and Voges-Proskauer (VP) test

MR-VP broth was used for the differentiation of bacteria by means of the methyl red and Voges-Proskauer reactions. This is a positive Voges-Proskauer reaction. These test method were done according to MR-VP Medium and MR-VP Broth (2008).

MR-VP broth medium

Composition of MR-VP broth medium (pH 7.0± 2.)

Components	Amount (gm/L)
Buffered Peptone	7.0
Dipotassium Phosphate	5.0
Dextrose	5.0

Procedure: All ingredients were dissolved in 1 liter of purified water and mixed thoroughly. If necessary, it was slightly heated to dissolve. Then it was dispensed and autoclaved at 121°C for 20 minutes.

Methyl red indicator

Components	Amount
Methyl red	0.1 g
95% ethyl alcohol	300 ml
Purified water	500 ml

Procedure: The methyl red indicator was prepared by dissolving 0.1 g of methyl red in 300 ml of 95% ethyl alcohol. Sufficient purified water was added to make 500 ml.

Voges-Proskauer test reagent

Reagent A:

Components	Amount
Alpha napthol	5 g
Absolute ethyl alcohol	95 ml

Reagent B:

Components	Amount
Potassium hydroxide	40 g
Creatine	0.3 g
Distil water	100 ml

Experimental Procedure

Methyl red test: 5 drops of methyl red indicator was added to an aliquot of the broth.

Voges-Proskauer test: 15 drops from the reagent A and 5 drops from the reagent B were added by dropper into 1 ml of broth culture. Then shake well after the addition of each reagent to aerate the sample.

Phenylalanine determination test

To demonstrate the ability of some organisms to remove the amino group ($-NH_2$) from amino acids.

Composition of phenylalanine agar (pH 7.3)

Component	Amount (gm/L)
Yeast extract	3.0
Dipotassium Phosphate	1.0
Sodium chloride	5.0
Bacto agar	12.0
Distilled water	1000 ml

Principle: Microorganism that contain deaminase enzymes capable of removing the amino group ($-NH_2$) from amino acids and other NH_2-containing chemical compounds. During this process the amino acid, under the auspices of its specific deaminase, will produce keto acids and ammonia as end products. In the experiment to follow, the amino acid phenylalanine will be delaminated by phelanineala ninedeaminize and convert to keto acid, phenylpyruvic acid and ammonia. The organisms are cultured on a medium incorporating phenylanine as the substrate. This reaction follows: if the organism processes phenylalanine deaminase, phenylpyruvic acid will be released into the medium and can be detected by the addition of a 10-12% ferric chloride solution to the surface of the medium. If a green color develops the enzymatic deamination of the substrate has occurred and is indicative of positive result, no color change indicates a negative result. The resultant green color produced upon the addition of ferric chloride ($FeCl_3$) is due to the formation of a keto acid.

Procedure: All ingredients were dissolved in 1 liter of purified water and mixed thoroughly. If necessary, it was slightly heated to dissolve. Then it was dispensed and autoclaved at 121°C for 20 minutes. Using sterile technique, inoculate each experiment organism into its appropriately labeled tube using a streak inoculation and incubated at 37°C for 24 to 48 hours.

Triple Sugar Iron (TSI) agar

Addition of sucrose in TSI Agar permits earlier detection of coliform bacteria that ferment sucrose more rapidly than lactose. Adding sucrose also aids the identification of certain gram-negative bacteria that could ferment sucrose but not lactose. Other basic understanding is TSI tube

contains butt (poorly oxygenated area on the bottom) slant (angled well oxygenated area on the top).

Composition of TSI test medium

Components	Amount (gm/L)
Peptic peptone	10
Casein peptone	10
Meat extract	3
Yeast extract	3
Lactose	10
Sacharose	10
Dextrose	1
NaCl	5
H_2SO_4	0.2
Sodium thyosulphate	0.
Phenol red	0.024
Agar	12

Media preparation: 64.7 g of media composition were suspended in 1 liter distilled water and heat until completely dissolved and autoclaved at 121^{0}C for 15 minutes and cool down to 4-5^{0}C. Then added urea solution 40% (TN1308) per liter of culture medium (final urea concentration: 2%). Mix well and fill into sterile tubes. Allow to set as slant agar with approximately equal length of butt and slant.

Procedure: A straight wire loop was used to inoculate TSI medium. The butt was first stabbed and then the slope was streaked in zigzag pattern and incubated at 35^{0}C for 24 hours. Cotton plugs of the tubes were kept loose. The bacteria therefore are exposed to both an anaerobic environment (butt) and an aerobic one (slant). Phenol red is present as an indicator. If the bacteria are non-fermenters, they can grow on the slant

by the aerobic degradation of protein components in the medium to alkaline products. The slant and the butt will remain red. If the bacteria can ferment dextrose, but not lactose, acid is produced in the slant and the butt and the medium turns yellow. The dextrose is used up, however, within 12 hours. Bacteria at the surface continue to grow by degrading proteins. By 18 to 24 hours, the alkaline end products cause the medium is the slant to revert to a red color. If the bacteria can ferment lactose as well as dextrose, the slant and butt will remain yellow after prolonged incubation. The high concentration of lactose keeps the slant acidic despite the production of alkaline products by protein degradation. TSI also contains sodium thiosulfate and ferrous sulfate as indictors of hydrogen sulfide production.

Coagulase Test

Coagulase test is used to differentiate *Staphylococcus aureus* (positive) from coagulase negetive *Staphylococcus* (CONS). Coagulase is an enzyme produced by *S. aureus* that converts (soluble) fibrinogen in plasma to (insoluble) fibrin. *Staphylococcus aureus* produces two forms of coagulase, bound and free. Rabbit plasma collection may be obtained by centrifuging blood to which 0.1% EDTA has been added as anticoagulage. Slide preparation to slide coagulase test firstly emulsify a staphylococcal colony in a drop of water on a clean glass slide with a minimum spreading as same as similar suspensions of control positive and negative strain were made to confirm the proper reactivity of the plasma. Then diped a flamed and cooled straight inoculating wire into the undiluted plasma at room temperature, withdraw and stir the adhering traces of plasma into the microbial suspension on the slide. Flame the wire and repeat for the control suspension. If a coarse clumping of cocci visible to the nacked eye within 10 seconds, read as positive and the

absence of clamping or any reaction taking more than 10 seconds to develop, considerate negative result. In this case were also done.

3.3 Pathogenicity test of the selected isolates

Pathogenicity is the ability of an organism, to produce and infectious disease in another organism. Pathogens are infectious agents that cause disease including bacteria, viruses, fungi, protozoa and higher parasites. The term used to describe the capacity of microbes to cause disease. To test of pathogenicity of selected isolates hemolytic activity test, virulence test on mice through direct ingestion of the isolates and hemagglutination were conducted.

3.3.1 Hemolytic activity test on blood agar

Tryptose blood agar base was used with blood for determining the hemolytic reactions of fastidious pathogenic microorganisms. Four different types of hemolysis on blood agar media can be described: (a). Alpha (α)- hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish decolonization of the medium. (b). Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony. (c). Gamma (γ) - hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium. (d). Alpha-prime (α′)-hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis. This test was carried out according to Harmon *et al.* (1995). Blood agar was prepared using following composition.

Component	Amount (gm/L)
Tryptose	10.0
Beef Extract	3.0
Sodium Chloride	5.0
Agar	15.0

Medium preparation

33 g of the components were suspended in 1L of purified water and mixed well. The heated for boiling for 1 minute to completely dissolve the powder and autoclaved at 121°C for 15 minutes. To prepare blood agar, aseptically added 5-10% sterile defibrinated blood in the medium and cooled at 45-50°C and mixed well. Then the medium poured into Petridis.

Procedure: Loopfull bacterial liquid cultures were streaked on blood agar. The cultural plates were incubated aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established. Then plates were examined for growth and hemolytic reactions after 18-24 and 48-hour incubation

3.3.2 Virulence test of selected bacteria on mice through direct ingestion

Virulence test of selected isolates was carried according to Rat Pyometra Model (Mikamo *et al.*, 1998) with slide modification. For this test a constant dose of McFarland OD 0.5 (1.5×10^8 cfu/ml) for each isolates were ingested in mice and sterile normal saline was ingested in control rat. The experiment period was continued for 20 days and weight loss was measured after 5 days' interval. Sickness, mortality of the mice was monitored for each treatment. Percentages (%) of weight losses after 20 days were determine for each rat by the following formula:

$$\text{Percentage (\%) of weight loss} = \frac{\text{Initial weight}(W_1) - \text{last weight}(W_2)}{\text{Initial weight}(W_1)} \times 100$$

3.3.3 Hemagglutination test of isolated bacteria

For haemagglutination test, it is necessary to test the settling pattern of the suspension of red blood cells. This involves mixing diluent with red blood cells and allowing the cells to settle. Normally haemagglutination is visible in microscope and it is the basis of haemagglutination tests to detect the presence of pathogenic bacteria.

Materials and Reagents: Red blood cells from mice were collected in hem buffer solution at appropriate pH. 20 mMTris-HCl was prepared by dissolving 0.24 gm of Tris base in about 50 ml of deionized distilled water. After adjusting the pH to 7.8 with concentrated HCl, the final volume was made up to 100 ml with deionized distilled water and dissolved in that buffer.

Procedure: The hem agglutination was performed in U-bottom polystyrene micro-titer plate as follows: 50 µl of 20 mMTris buffer were poured into serially according to samples. 50 µl of each bacterial solution in Tris-HCl buffer were added in in micro-titer plate according to $A_1 - A_6$, $B_1 - B_6$, $C_1 - C_6$, $D_1 - D_6$, $E_1 - E_6$ no. well of micro-titer plate with the suspension of RSVE-185, RKFE-227, BAFM-417, SMRM-651 and SMRS-671 isolates, respectively. Bacterial suspension and buffer were mixed well. One-fold serial dilution was carried out from 1:1 diluent from wells no A_1 to E_1 down to A_6 to E_6. In case of control containing 50 µl of 20 mMTris buffer were taken instead of bacterial suspension and 50 µl blood cell suspensions and suspensions were mixed in F_1 to F_6 No well. The mixture in the titers plate was mixed well by gentle shaking with shaker continuously for 15 minutes and wait until 30 minutes. After that the sediment erythrocytes were gently mixed with the supernatant and one drop of this suspension was examined under microscope (Plate-4.7). The agglutination activity was expressed as visible agglutination could be detected.

Chapter Four

Results

RESULTS

4.1. Survey of different restaurants and street food vans and vendors of Rajshahi Metropolis

In the present study different restaurants and street food vans and vendors of six hot spots of Rajshahi Metropolis were surveyed during March 2015 to February 2016. The survey were mainly conducted to ascertain the socio economic condition, education quality and hygiene practice of food sellers and as well as their waste management system.

Selection of survey areas

Total sixty five restaurants and twenty five street food vans and vendors of six locations namely Laxmipur, Court, Naodapara, Sahebazer Binodpur, RU Campus in Rajahshi Metropolis were surveyed (Fig.4.1).

The main considerations in selecting the study areas were as below:

(a) Large number of restaurants were raised in these areas;
(b) This type of study was not conducted previously;
(c) Well-communication system of the restaurants;
(d) Co-operation from the restaurant owners was highly satisfactory; and
(e) Lacking of personnel awareness of the restaurant workers.

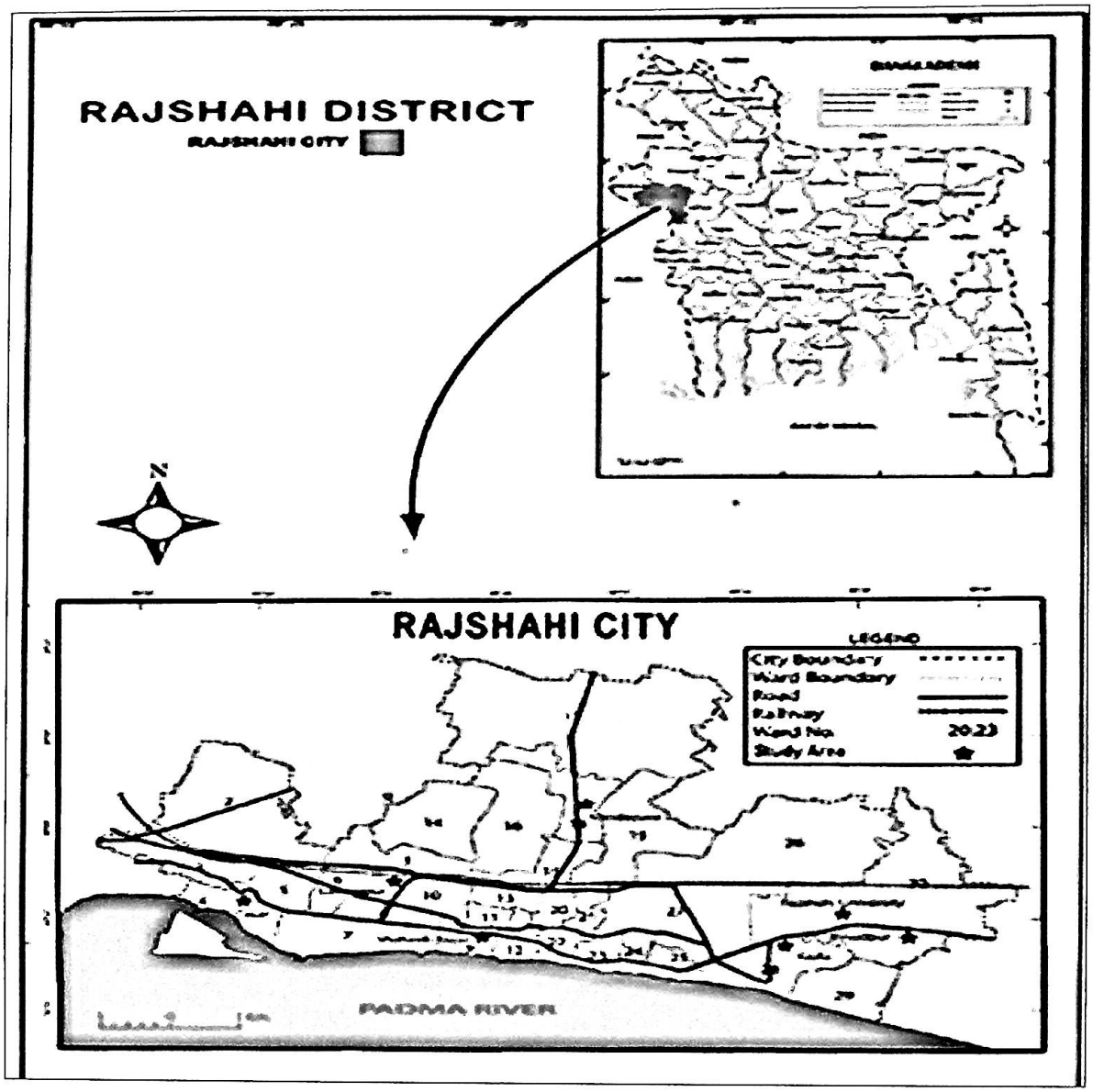

Fig 4.1. Map showing different locations of o sampling area (Yellow marks indicator the sampling area)

The survey data were analyzed in certain points and compared with different locations which are given below:

Analysis of survey data on restaurants condition

The restaurants conditions of the surveyed areas were assessed on the characteristics of darkness, damp, proper aeration and light and the results are presented in Table 4.1. The maximum restaurants of court areas were dark and dirty compare to others. The restaurant of Saheb Bazar were in better condition than Binodpur, Naodapara and Laxmipur. Proper aerotion and light were observed in the restaurant of Saheb Bazaar areas but the maximum restaurants on Laxmipur and Naodapara were not in good condition.

Analysis of survey data on sanitation status

The sanitation condition of the restaurants of the surveyed areas were assessed according to cleanliness, sanitizer used and waste management system of the restaurants and the results are presented in Table 4.1. Most of the restaurants were clean regularly with normal detergent powder. No sanitizer was used. Waste management system of the restaurants of Saheb Bazaar were better while RU campus, Binodpur, Laxmipur were good and court and Naodapara areas were poor (Table 4.1). In overall perception, it was observed that the 30% restaurants of Shaheb Bazar, 20% of RU campus, 15% of Binodpur, 7.6 % of Laxmipur were in best conditions but none of the restaurants of Court and Naodapara areas were found in best condition.

Table 4.1. Surveillance of restaurants of Rajshahi Metropolis on restaurant condition and sanitation status during March 2015 to February 2016.

Location	Name of restaurant	Food item Sold	Restaurant condition			Sanitation status			Overall perception
		Rice, Cook Dal. Fish, Vegetables, & Chicken	Dark & dirty	Damp	Proper aeration & light	Cleanliness	Used sanitizer/ detergent	Waste management systems	
RU Campus	Kalam	,,	-	+	-	-	+	-	-+
	Selim	,,	-	+	-	-	+	-	-
	Sagor	,,	-	+	-	++	++	++	++
	Mohon	,,	-	+	-	-	+	-	-+
	Jolil	,,	-	+	-	-	+	-	-+
	Green Vally	,,	+	+	+	++	++	++	++
	Silsila	,,	+	+	+	+++	+++	+++	+++
	Cafeteria	,,	+	+	+	+++	+++	+++	+++
	Milon	,,	-	+	-	-	+	-	-+
Binopdpur	Sadek	,,	-	-	-	++	++	++	++
	ShilpiBiriyani	,,	-	+	-	++	++	++	++
	Sirajul	,,	-	-	-	-	+	-	- +
	Latif	,,	-	-	-	-	+	-	- +
	New hotel & restaurant	,,	+	+	-	-	+	-	- +
	ZilapiBhandar	,,	+	+	-	-	+	-	- +
	Out back	,,	-	+	-	++	++	++	++
	Delounge		+	+	+	++	++	++	++
	Siyamoon		+	+	+	+++	+++	+++	+++
	Royel Hotel	,,	-	+	-	-	+	-	- +
	Alamin Hotel	,,	+	-	-	-	+	-	- +
	Maisha	,,	-	-	-	-	+	-	- +
	VatVorta	,,	+	+	-	+++	+++	+++	+++
	HajiBiriani	,,	+	-	-	++	++	++	++
Saheb Bazar	BidduSt	,,	+	+	+	+++	+++	+++	+++
	Keya	,,	-	-	-	++	++	++	++
	Rahamania	,,	+	+	+	+++	+++	+++	+++
	Selim	,,	-	-	-	++	++	++	++
	Saidur	,,	-	-	-	++	++	++	++
	Mazeda	,,	-	-	-	-	+	-	- +
	Café amar	,,	-	+	-	-	+	-	- +
	Chilis	,,	+	+	+	+++	+++	+++	+++
	Satl met	,,	+	+	+	++	++	++	++
	Himel	,,	+	+	-	-	+	-	- -
	Anghur	,,	+	+	-	-	++	-	- -
	Memory	,,	+	+	-	++	++	++	+ +
	Hilsha	,,	+	+	-	-	+	-	- +
	Frizzle	,,	+	+	+	-	+	-	- +
	Flavours	,,	+	+	+	+++	+++	+++	+ ++
	Bindu	,,	+	+	+	+++	+++	+++	+++
	Ghost raider	,,	+	+	+	++	++	++	
	Dallus	,,	+	+	+	+++			
	Mirza hotel	,,	-	-	-	-			

Location	Name of restaurant	Food item Sold	Restaurant condition			Sanitation status			Overall perception
		Rice, Cook Dal. Fish, Vegetables, & Chicken	Dark & dirty	Damp	Proper aeration & light	Cleanliness	Used sanitizer/ detergent	Waste management systems	
	Oder up	,,	+	+	+	-	+	-	- +
Court	Bhaibhai	,,	+	+	+	-	+	-	- +
	Joy Ma	,,	+	+	-	-	+	-	- +
	Jony	,,	-	-	-	-	+	-	- +
	Maiuddin	,,	-	+	-	-	+	-	- -
	Khabar hotel	,,	+	+	+	++	++	++	++
Laxmipur	Café raj	,,	+	+	+	++	++	++	++
	NurNishan	,,	+	+	-	++	++	++	++
	Ramzan	,,	-	+	-	-	+	-	- +
	Star	,,	-	-	-	++	++	++	++
	Tripty	,,	+	+	+	+++	+++	+++	+++
	Eman	,,	+	-	-	++	++	++	++
	Kutum	,,	-	+	-	-	+	-	- +
	Al-modina	,,	-	+	-	-	+	-	- +
	Sharminerk habar	,,	-	+	-	-	+	-	- +
	Kisan	"	+	+	-	++	++	++	++
	Sikdar	,,	-	-	-	-	+	-	- +
	Padma	,,	-	-	-	-	+	-	- +
	Mustak	,,	-	-	-	-	+	-	- +
Noudapara	Eyasin	,,	+	-	+	++	++	++	++
	Ma	,,	+	+	-	-	+	-	-+
	Tusty	"	-	-	-	++	++	++	++
	Mouchak	,,	-	+	-	-	+	-	- +

Note: - = Poor , -+ =Good, ++ = Better, +++ =Best .

Analysis of survey data on socioeconomic status and education level of restaurants vendors

The socioeconomic status and education level of the restaurant vendors were studied and the results are presented in Table 4.2. From the survey data it was observed that majority (100%) of restaurant vendors were male. Age ranges of the majority (33.84%) of the vendors were 25-29 years and next of 32.31% vendors were 40-49 years old and 26.15% restaurants were below 25 years and only 7.69% were 50 plus. The education level of the restaurant vendors of Rajshahi Metropolis were also recorded. The maximum (42%) restaurant vendors were passed SSC, 29% were passed primary, 23% were passed H.S.C and only 6% restaurant vendors were illiterate. The majority (74%) of vendors take it as main occupation. They have more or less 5 to 20 years of experience in this profession. The majority (32.30%) of restaurants vendors earned 26000 to 30000 taka monthly and minimum (8% vendors) had monthly income 10,000 to 15000 taka.

Table 4.2. Socioeconomic status of restaurant vendors

Characteristics		Frequency (%) N=65
Gender		
Male		100
Female		0
Age Group		
<25		17 (26.15)
25-39		22 (33.84)
40-49		21 (32.31)
50 plus		5 (7.69)
Education Level		
Illiterate		4 (6.15)
Primary		15 (23.08)
S.S.C		27 (41.54)
H.S.C		19 (29.23)
Major Income Source for the Vendors		
Yes		48 (73.85)
No		17 (26.15)
Experience in the Occupation		
> 5 years		18 (27.69)
5-9		25 (38.46)
10-19		15 (23.08)
20 above		7 (10.77)
Members of Cooperative		
Yes		15 (23.08)
No		50 (76.92)
Employees Engaged in the unit		
None		0
1-3		15 (23.08)
3 & over		50 (76.92)
Average Monthly Income(Taka)		
10000-15000		5(7.69)
16000-2,0000		7 (10.77)
210000-25000		18 (27.69)
26000-300000		21 (32.30)
Above 50000		14(21.5)

Analysis of hygienic and sanitation status of restaurants vendors and restaurants.

The hygienic and sanitation status of the restaurant vendors were studied and the results are presented in Table 4.3. From the survey data it was observed that most of the restaurant vendors (58%) had not knowledge about food hygiene and 39% restaurant vendors had health certificate. Most of them knew regular hand washing practices with soap and water (85%), proper covering of food (92.30%), proper cleaning of sale surfaces (23.07%) and storage of food in refrigerator (76.92%.). Hand washing before and after serving was practiced by (23.07%) respondents, (30.77%) used aprons and none of respondents covered hair with caps or used gloves while serving food. They used tube-well and supply water for drinking and washing purpose. 100% of the restaurants used detergent for cleaning of utensil. The utensils used in serving food were considered clean in majority (53.84%) cases. There were 48% restaurant remained in poor sanitation condition while only 17% were in better condition. None of the respondents have any training about food safety and majority (92.30%) were not aware of food safety law.

Table 4.3. Hygienic and sanitation status of restaurant vendors

Characteristics		Frequency(%) N=65
Food Hygiene / Safety Knowledge		
Yes		27 (41.54)
No		38 (58.46)
Health Certificate Posses		
Yes		25(38.46)
No		40 (61.54)
Aprons used		
Yes		20 (30.77)
No		45 (69.23)
Caps/gloves used		
Yes		0
No		65 (100)
Source of water		
Tube well		18 (27.69)
Supply		47 (72.31)
Hand wash before and after serving food		
Yes		15 (23.07)
No		50(76.92)
Sanitation Level		
Poor		31 (47.69)
Good		18 (27.69)
Better		11 (16.92)
Regular cleaning (utensil)		
Water		0
Detergent powder		65 (100)
Storage of food in refrigerator		
Yes		50 (76.92%)
No		15(23.07%)
Utensils used		
Clean		35(53.84)
Dirty		30(46..15)

Characteristics		Frequency(%) N=65
Regular hand washing practices		
Water		10(15.38%)
Soap and water		55(84.61)
Covering of food		
Yes		60(92.30)
No		5(7.69)
Cleaning of sale surfaces		
Regular		15(23.07)
Irregular		50(76.92)
Food safety training		
Yes		0
No		65
Food safety law		
Known		5(7.69)
Unknown		60(92.30)
Waste management system		
Poor		31(47.69)
Good		19(29.23)
Better		15(23.07)

N = Total individual

Analysis of survey data on socio-economic condition, hygiene and sanitation status of street food vendors

The socio-economic condition, hygiene and sanitation status of street food vendors were studied and the results are presented in Table 4.4. From the survey data it was observed that majority (100%) of street vendors were male. Age ranges of the majority (60%) of the street vendors were 36-45 years and next of 20% vendors were 46-55 years old, 12% were 26-35 years and minimum 8% restaurants were below 15-25 years. The education level of the restaurant vendors of Rajshahi Metropolis were also recorded. The maximum (60%) restaurant vendors were passed JSC, 20% were passed primary, 12% were passed S.S.C and only 8% street vendors were illiterate. The majority (80%) of vendors took it as main occupation. The majority (52%) of street vendors earned 11000 to 15000 taka monthly and minimum (12% vendors) had monthly income 5,000 to 10000 taka. Most of the street food vendors (80%) had no knowledge about food hygiene. None of the vendors (100%) used apron, gloves while selling RTE food and did not covered food with polythene.

Table 4.4 Socioeconomic, hygienic and sanitation status of of street vendors.

Characteristics	Frequency (%) N=25
Gender	
Male	100
Female	0
Age group	
15-25	2 (8)
26-35	3 (12)
36-45	15 (60)
46-55	5 (20)
Level of Education	
Illiterate	2(8)
(≤)Primary	5 (20)
(≤)J.S.C	15 (60)
(≤)S.S.C	3 (12)
Major income sources	
Yes	20 (80)
No	5 (20)
Food hygiene knowledge	
Yes	5 (20)
No	25 (80)
Use of aprons	
Yes	0 (100)
No	25 (0)
Use of cap	
Yes	0(100)
No	25 (0)
Use of gloves	
Yes	0(100)
No	25 (0)
Food covered with polythene	
Yes	
No	25 (0)
Monthly income (Taka)	
5000-10000	3 (12)
11,000-15,000	13 (52)
16,000-20,000	7 (28)
21,000-25,000	2 (8)

Plate 4.1. Photographs showing different types of RTE restaurants and food vending carts in surveyed areas

Plate 4.2.Photographs showing waste management systems of street food vending stall/van (A-C) and restaurants (D-F)

Recommendation

Sanitation and hygienic practices of restaurants and street food vendor's of six hot spots of Rajshahi Metropolis were served. On the basis of survey results some exclusive and key points were including as recommendation which are given below.

1. The restaurants and street vendors should wear apron, gloves, caps etc. during the serving time.

2. They should used sanitizer properlyfor cleaning of utensil and vending site.

3. By increasing the awareness of personal hygiene knowledge of RTE foods vendors, contamination rate or disease transmission can be reduced.

4. The restaurant owner should emphasis on cleanliness and sources of drinking water (tube-well) to avoid microbial contamination.

5. The concerted efforts are needed to improve the sanitary practices of street vended foods and to improve the livelihood of street food vendors.

6. Regular monitoring of microbial quality of each RTE food items are needed according to vending site and region.

7. Regular training or inspection of street/Restaurant for good food handling practices should be mandatory.

8. Food vendors should be encouraged to operate designated places and local authorities should provide the necessary infrastructure in order to improve the safety of street-vended foods.

9. Print and electronic media can contribute to campaign regarding this.

10. Our government should provide national or regional microbial guidelines to the local authority to regular checking.

11. Our government should take step for strong monitoring and enforcement authority should visit the market places regularly.

Finally by integration of Government and public authorities and increasing of awareness of food vendors regarding hygiene practices the concern sector will improve.

4.2 Microbial quality assessment of RTE foods

In the present study different RTE food samples were collected from restaurants and street food vendors of six hot spots of Rajshahi Metropolis and surrounding areas during March 2015 to February 2016. These food samples were subjected for microbial quality assessment. For this purpose Nutrient agar medium was used to total viable count (TVC), MacConkey agar (MA) was used to total coliform count, eosin metheline blue (EMB) was used to total *E. coli* count, *Salmonella-Shigella* (SS) agar medium was used to total *Salmonella* count and Manitol salt (MS) agar medium used for total *Staphylococcus* count following conventional culture plate method and the result are given bellow:

4.2.1 Total viable count of RTE food samples on NA medium

Seven RTE food items namely boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti were selected for TVC on NA medium and the results are presented in Table 4.5, Plate- 4.2(A). In boiled rice, the colony forming unit (cfu) of total viable bacteria were the ranged as 3.4×10^{6} to 6.2×10^{12} cfu/gm and hundred percent samples contained $\geq 10^{6}$ cfu/gm.

Vegetable curry samples were placed on nutrient agar (NA) medium and the colony forming unit (cfu) of total viable bacteria were counted. The ranges of cfu were counted as 1.24×10^{7} to 9×10^{11} cfu/gm. Hundred percent samples contained $\geq10^{6}$ cfu/gm.

Chicken curry samples were placed on nutrient agar (NA) medium and the colony forming units (cfu) of total viable bacteria were counted. The

ranges of cfu were recorded as 3.4×10^{7} to 8.8×10^{12} cfu/gm. Hundred percent samples contained $\geq10^{6}$ cfu/gm.

Fish curry samples were placed on nutrient agar (NA) medium and the colony forming unit (cfu) of total viable bacteria was counted. The ranges of cfu were recorded as 2.0×10^{7} to 6.5×10^{12} cfu/gm. Hundred percent samples contained $\geq10^{6}$ cfu/gm.

Cooked dal samples were placed on nutrient agar (NA) medium and the colony forming unit (cfu) of total viable bacteria was counted. The ranges of cfu were recorded as 1.3×10^{6} to 4.6×10^{12} cfu/gm. Hundred percent samples contained $\geq10^{6}$ cfu/gm.

Chatpoti samples were placed on nutrient agar (NA) medium and the colony forming unit (cfu) of total viable bacteria was counted. The ranges of cfu were recorded as 34.7×10^{5} to 51.4×10^{10} cfu/gm. Hundred percent samples contained $\geq10^{6}$ cfu/g.

Fuchka samples were placed on nutrient agar (NA) medium and the colony forming unit (cfu) of total viable bacteria was counted. The ranges of cfu were recorded as 41.7×10^{6} to 52.8×10^{12}cfu/gm. Hundred percent samples contained $\geq10^{6}$ cfu/g.

4.2.2 Total coliforms count of RTE food samples on MacConkey medium

Seven RTE food samples namely boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti were selected for total coliforms on MacConkey agar medium and the results are presented in Table 4.6, Plate 4.2(B). In boiled rice, the ranges of cfu of total coliforms were recorded as 1.42×10^{5} to 9.9×10^{9} cfu/gm. Coliform contamination was detected in about hundred percent of the samples with $\geq10^{4}$ cfu/gm.

In vegetable curry, MacConkey medium was used for total coliforms count. The ranges of total coliforms were recorded as 6×10^5 to 4×10^{10} cfu/gm. Hundred percent samples were contaminated by total coliforms with $\geq10^4$ cfu/gm.

In chicken curry, MacConkey medium was used for total coliforms count and the ranges of total coliforms were recorded as 1.1×10^6 to 5×10^{11} cfu/gm. Hundred percent samples were contaminated by coliforms with $\geq10^4$ cfu/gm.

In fish curry, MacConkey medium was used for total coliforms count and the ranges of total coliforms were recorded as $8.4\times10^6$to 4.8×10^{11} cfu/gm. Coliform contamination was detected in about hundred percent of the samples with $\geq10^4$ cfu/gm.

In cooked dal, MacConkey medium was used for total coliforms count and the ranges of total coliforms were recorded as 8.4×10^6 to 6.3×10^{10}cfu/gm. Hundred percent of samples were contaminated with $\geq10^4$ cfu/gm.

In chatpoti, MacConkey medium was used for total coliforms count and the ranges of total coliforms were recorded as 5.2×10^3 to 8×10^6 cfu/gm. Coliform contamination was detected in about 25-50% of the samples with $\geq10^4$ cfu/gm.

In Fuchka, MacConkey medium was used for total coliforms count and the ranges of total coliforms were recorded as 2.1×10^4 to 12.3×10^8 cfu/gm. Coliform contamination was detected in about 25-75% of samples with $\geq10^4$ cfu/gm.

4.2.3 Total *E. coli* count of RTE food samples on EMB medium

Seven RTE food samples i.e. boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti were selected for total *E. coli* count and the results are presented in Table 4.7, Plate 4.2(C)). In boiled rice, the samples were placed on EMB medium and the cfu of *E. coli* were counted. The ranges of cfu of total *E. coli* were recorded as 0 to 5.2×10^3 cfu/gm. 17-100% samples were contaminated by *E. coli* with $\geq10^2$ cfu/gm.

In vegetable curry, EMB medium was used for total *E. coli* count and the ranges of cfu of total *E. coli* were recorded as 1×10^2 to 4×10^6 cfu/gm while 17-100% samples were contaminated by *E. coli* with $\geq10^2$cfu/gm.

In chicken curry, EMB medium was used for total *E. coli* count and the ranges of cfu of total *E. coli* were recorded as 1.12×10^2 to 5.2×10^5 cfu/gm and 50-100% samples were contaminated with $\geq10^2$ cfu/gm.

In fish curry EMB medium was used for total *E. coli* count and the ranges of cfu of total *E. coli* were recorded as 0 to 9.4×10^4 cfu/gm and 0-100% samples were contaminated with $\geq10^2$ cfu/gm.

In cooked dal, EMB medium was use for total *E. coli* count and the ranges of cfu of total *E. coli* were recorded as 0 to 6.3×10^6 cfu/gm. 0-100% samples were contaminated by *E. coli* with $\geq10^2$ cfu/gm. *E. coli* was not detected in cooked dal samples in Court and Naodapara areas.

In chatpoti , EMB medium was used for total *E. coli* count and the ranges of total *E. coli* were recorded as 1.4×10^3 to 4.8×10^6 cfu/gm. Hundred percent samples were contaminated by *E. coli* with $\geq10^2$ cfu/gm.

In fuchka EMB medium was used for total *E. coli* count and the ranges of *E. coli* were recorded as 2.9 $\times 10^4$ to 5.1×10^7 cfu/gm. Hundred percent samples were contaminated by *E. coli* with $\geq 10^2$ cfu/gm.

4.2.4 Total *Salmonella* count of of RTE food samples on SS medium

Seven RTE food samples i.e. boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti were selected for total *Salmonella* count and the results are presented in Table 4.8, Plate 4.2(D). In boiled rice, SS medium was used for total *Salmonella* count and the ranges of cfu of total *Salmonella* were 0 to 6.8×10^2 cfu/gm. 0 to 100% percent samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm.

In vegetable curry, SS medium was used for total *Salmonella* count and the ranges of cfu of total *Salmonella* were 0 to 2.8×10^2 cfu/gm. 0 to 84% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm.

In fish curry, SS medium was used for total *Salmonella* count and the ranges of cfu of total *Salmonella* were counted as 0 to 6.2×10^2 cfu/gm. 0 - 50% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm.

In chicken curry, SS medium was used for total *Salmonella* sp. count and the ranges of cfu of total *Salmonella* were counted as 0 to 8.4×10^5 cfu/gm. 17-100% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm.

In cooked dal, SS medium was used for total *Salmonella* sp. count and the ranges of cfu of total *Salmonella* were counted as 0 to 9.2×10^4 cfu/gm. 17-100% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm.

In fuchka, SS medium was used for total *Salmonella* sp. count and the ranges of cfu of total *Salmonella* were counted as 0 to 5.1×10^3 cfu/gm. 25 -50 % samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm.

In chatpoti, SS medium was used for total *Salmonella* sp. count and the ranges of cfu of total *Salmonella* were counted as 0 to 5.1×10^2. 25 - 100% samples were contaminated by *Salmonella* with $\geq10^1$ cfu/gm.

4.2.5 Total *Staphylococcus* count of of RTE food samples on MS medium

Seven RTE food items like boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti were selected for total *Staphylococcus* count and the results are presented in Table 4.9, Plate 4.2(E). In boiled rice MS medium was used for *Staphylococcus* sp. count and the ranges of cfu of total *Staphylococcus* were recorded as 0 to 6.1×10^7 cfu/gm. 0 to 34% samples were contaminated by *Staphylococcus* with $\geq10^6$ cfu/gm.

In vegetable curry, MS medium was used for total *Staphylococcus* count and the ranges of cfu were recorded as 0 to 3.2×10^6 cfu/gm. 0 to 34% samples were contaminated by *Staphylococcus* with $\geq10^6$ cfu/gm.

In chicken curry, MS medium was used for total *Staphylococcus* count and the ranges of cfu of total *Staphylococcus* were recorded as 2×10^2 to 9.2×10^5 cfu/gm. No samples were contaminated with $\geq10^6$ cfu/gm.

In fish curry, MS medium was used for total *Staphylococcus* count and the ranges of cfu of total *Staphylococcus* were recorded as 6×10^2 to 6.4×10^5 cfu/gm. No samples were contaminated with $\geq10^6$ cfu/gm.

In cooked dal, MS medium was used for *Staphylococcus* count and the ranges of cfu of total *Staphylococcus* were recorded as 2×10^3 to 3×10^6 cfu/gm. 0 to 50% samples were contaminated by *Staphylococcus* with $\geq10^6$ cfu/gm.

In chatpoti, MS medium was used for *Staphylococcu.* count and the ranges of cfu of total *Staphylococcus* were recorded as 2.5×10^3 to 9×10^7 cfu/gm. 25-100% samples were contaminated by *Staphylococcus* with $\geq10^6$ cfu/gm.

In fuchka, MS medium was used for *Staphylococcus.* count and the ranges of cfu of total *Staphylococcus* were recorded as 4.4×10^4 to 6.0×10^9 cfu/gm. 50-100% samples were contaminated by *Staphylococcus* with $\geq10^6$ cfu/gm.

Plate 4.3. Photographs showing different selected restaurants boiled rice (A), cooked dal (B), vegetable curry (C), fish curry (D) and chicken (E) and street food fuchka (F) and chatpoti (G) items sold in Rajshahi Metropolis

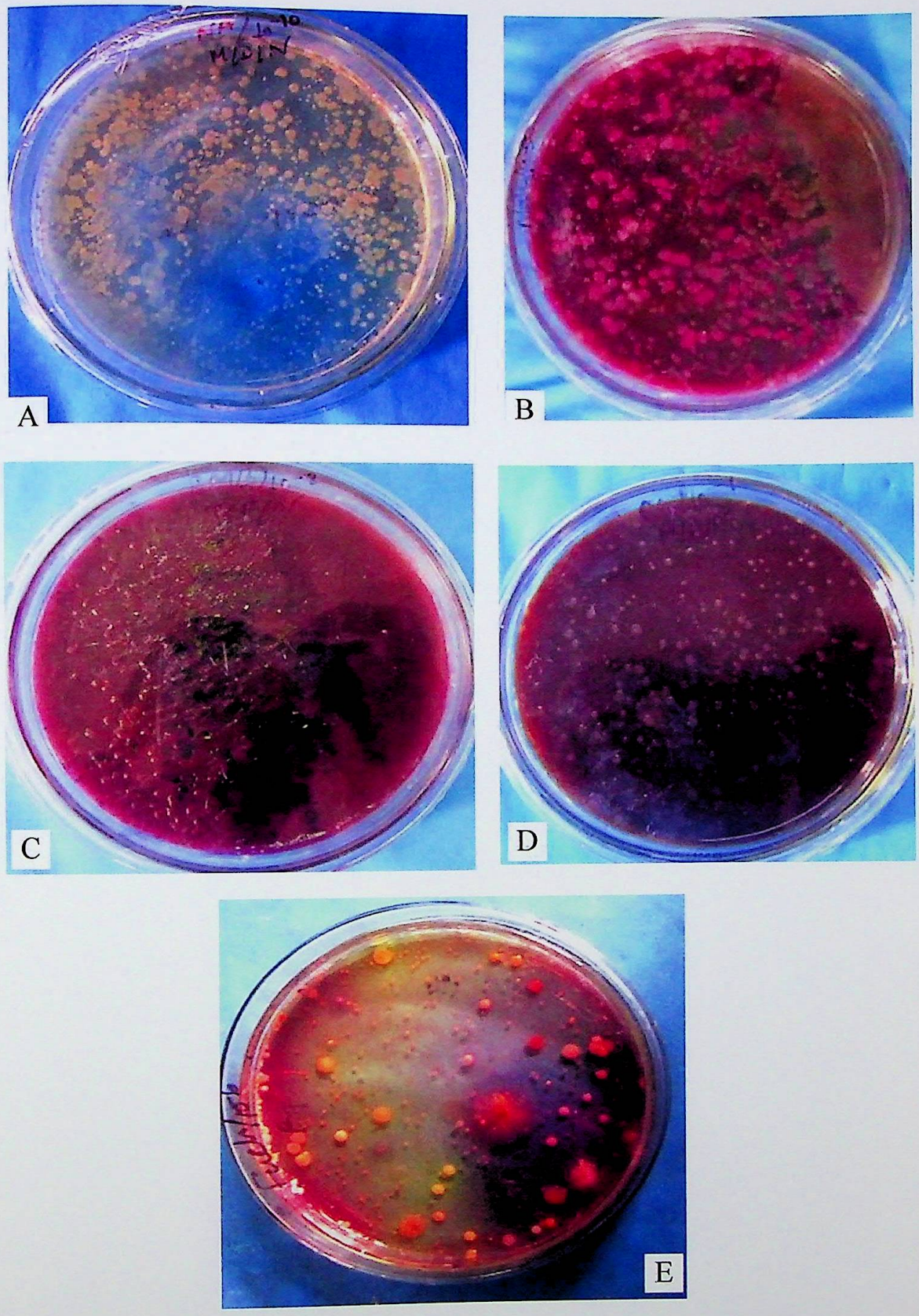

Plate 4.4. Photographs showing bacterial colonies on different medium: NA (A), MacConkey agar (B), EMB agar (C), SS agar (D) and MS agar medium (E)

Table 4.5. Total viable count (TVC) of RTE food samples on NA medium

Food items	Sampling area	Ranges of cfu/gm	Samples contaminated with $\geq 10^6$cfu/gm
Boiled rice	Laxmipur	4.6×10^{11} to 6.2×10^{12}	100%
	RU Campus	5.6×10^{6} to 4×10^{11}	100%
	Court	8.4×10^{10} to 5.2×10^{12}	100%
	Binodpur	5.2×10^{11} to 4.4×10^{12}	100%
	Naodapara	2×10^{11} to 1.46×10^{12}	100%
	Saheb Bazar	3.4×10^{6} to 4.8×10^{9}	100%
Vegetable curry	Laxmipur	5×10^{7} to 9×10^{11}	100%
	RU Campus	2.6×10^{9} to 5×10^{11}	100%
	Court	1×10^{8} to 3.4×10^{10}	100%
	Binodpur	3.6×10^{9} to 6.2×10^{11}	100%
	Naodapara	3.6×10^{10} to 6.2×10^{11}	100%
	Saheb Bazar	1.24×10^{7} to 2×10^{10}	100%
Chicken curry	Laxmipur	6×10^{10} to 8.8×10^{12}	100%
	RU Campus	2.6×10^{8} to 5×10^{11}	100%
	Court	4.8×10^{8} to 2.8×10^{11}	100%
	Binodpur	3.6×10^{10} to 6.4×10^{12}	100%
	Naodapara	4×10^{11} to 4×10^{12}	100%
	Saheb Bazar	3.4×10^{7} to 1.36×10^{9}	100%
Fish Curry	Laxmipur	3×10^{10} to 6.5×10^{12}	100%
	RU Campus	4.6×10^{10} to 5×10^{11}	100%
	Court	1.6×10^{8} to 3×10^{10}	100%
	Binodpur	4×10^{11} to 4.4×10^{12}	100%
	Naodapara	4×10^{10} to 4×10^{12}	100%
	Saheb Bazar	2×10^{7} to 1.14×10^{9}	100%
Cooked Dal	Laxmipur	5×10^{11} to 4.6×10^{12}	100%
	RU Campus	4×10^{10} to 4×10^{11}	100%
	Court	1.28×10^{8} to 2.4×10^{10}	100%
	Binodpur	4.52×10^{10} to 2.2×10^{12}	100%
	Naodapara	4×10^{11} to 3.6×10^{12}	100%
	Saheb Bazar	1.3×10^{6} to 9.6×10^{8}	100%
Fuchka	Laxmipur	41.7×10^{6} to 53.8×10^{10}	100%
	RU Campus	36.1×10^{7} to 51.2×10^{10}	100%
	Court	34.9×10^{8} to 51.3×10^{11}	100%
	Binodpur	35.6×10^{6} to 52.6×10^{10}	100%
	Naodapara	36.8×10^{8} to 52.2×10^{10}	100%
	Saheb Bazar	25.6×10^{8} to 52.8×10^{12}	100%
Chatpoti	Laxmipur	33.8×10^{8} to 51.4×10^{10}	100%
	RU Campus	34.7×10^{6} to 47.9×10^{8}	100%
	Court	33.1×10^{6} to 42.8×10^{9}	100%
	Binodpur	36.1×10^{6} to 48.0×10^{8}	100%
	Naodapara	38.7×10^{6} to 50.8×10^{9}	100%
	Saheb Bazar	34.7×10^{5} to 48.6×10^{8}	100%

Table 4.6. Total coliforms count of RTE food sample on MacConkey medium

Food items	Sampling area	Ranges of cfu/gm	Samples contaminated with ≥ 10^4 cfu/gm
Boiled rice	Laxmipur	3.6×10^6 to 9.9×10^8	100%
	RU Campus	2.6×10^6 to 3.18×10^8	100%
	Court	3×107 to 4.8×108	100%
	Binodpur	1.44×10^6 to 5.92×10^8	100%
	Naodapara	2.56×10^6 to 6×10^7	100%
	Saheb Bazar	1.42×10^5 to 5.2×10^7	100%
Cooked Dal	Laxmipur	1.3×10^8 to 6.3×10^{10}	100%
	RU Campus	7×10^7 to 2×10^9	100%
	Court	1.6×10^8 to 2.4×10^{10}	100%
	Binodpur	1.9×10^8 to 5.5×10^{10}	100%
	Naodapara	2.16×10^8 to 7×10^{10}	100%
	Saheb Bazar	8.4×10^6 to 8.8×10^8	100%
Vegetable curry	Laxmipur	1.12×10^8 to 4×10^{10}	100%
	RU Campus	1.4×10^7 to 6.4×10^8	100%
	Court	4×10^8 to 1.04×10^{10}	100%
	Binodpur	4×10^8 to 3.4×10^{10}	100%
	Naodapara	6.2×10^8 to 4.2×10^{10}	100%
	Saheb Bazar	6×10^5 to 5.4×10^6	100%
Fish Curry	Laxmipur	4×10^8 to 4.8×10^{11}	100%
	RU Campus	4.8×10^7 to 7.8×10^8	100%
	Court	4.94×10^6 to 4.8×10^9	100%
	Binodpur	5.24×10^8 to 4.8×10^{10}	100%
	Naodapara	3.6×10^6 to 3.2×10^{10}	100%
	Saheb Bazar	8.4×10^6 to 7.80×10^8	100%
Chicken curry	Laxmipur	1.08×10^8 to 5×10^{11}	100%
	RU Campus	1.24×10^7 to 2.4×10^9	100%
	Court	6.2×10^7 to 6.4×10^9	100%
	Binodpur	1.94×10^8 to 3.4×10^{10}	100%
	Naodapara	1.68×10^8 to 4×10^9	100%
	Saheb Bazar	1.1×10^6 to 8×10^8	100%
Fuchka	Laxmipur	2.1×10^4 to 5.1×10^6	50%
	RU Campus	6.1×10^4 to 9.3×10^6	25%
	Court	7.8×10^4 to 12.1×10^6	75%
	Binodpur	5.5×10^4 to 6.5×10^6	25%
	Naodapara	5.1×10^4 to 6×10^6	75%
	Saheb Bazar	7.6×10^6 to 12.3×10^8	75%
Chatpoti	Laxmipur	5.1×10^4 to 8×10^6	50%
	RU Campus	6.5×10^3 to 6.8×10^6	50%
	Court	5.3×10^3 to 2.2×10^6	50%
	Binodpur	5.5×10^3 to 11.2×10^6	25%
	Naodapara	6.9×10^3 to 8.6×10^5	50%
	Saheb Bazar	5.2×10^3 to 2.7×10^5	25%

Table 4.7. Total *E .coli* count of RTE food samples on EMB medium

Food items	Sampling area	Ranges of cfu/gm	Samples contaminated with $\geq 10^2$ cfu gm
Boiled rice	Laxmipur	2.0×10^3 to 6.6×10^4	100%
	RU Campus	1.4×10^3 to 1.08×10^4	100%
	Court	0 to 6×10^3	17%
	Binodpur	5×10^3 to 4.84×10^4	100%
	Naodapara	3×10^2 to 5.2×10^3	50%
	Saheb Bazar	0 to 1.9×10^3	17%
Vegetable curry	Laxmipur	6×10^3 to 4×10^6	100%
	RU Campus	2.3×10^3 to 3×10^4	100%
	Court	1.8×10^2 to 4.23×10^4	17%
	Binodpur	1.04×10^3 to 6.1×10^4	100%
	Naodapara	2.24×10^3 to 3×10^4	100%
	Saheb Bazar	1×10^2 to 1.24×10^4	67%
Cooked Dal	Laxmipur	4×10^4 to 6.3×10^6	100%
	RU Campus	2×10^3 to 4×10^6	100%
	Court	0	0%
	Binodpur	1.7×10^3 to 5×10^4	100%
	Naodapara	0 to 8.2×10^3	17%
	Saheb Bazar	1.2×10^2 to 4×10^5	67%
Fish Curry	Laxmipur	6×10^3 to 9.4×10^4	100%
	RU Campus	9.2×10^3 to 1.44×10^4	100%
	Court	0	0%
	Binodpur	1.3×10^1 to 2×10^2	100%
	Naodapara	2×10^3 to 8.2×10^4	100%
	Saheb Bazar	2×10^3 to 1.44×10^4	100%
Chicken curry	Laxmipur	2.4×10^3 to 5.2×10^5	100%
	RU Campus	1.3×10^3 to 3×10^4	100%
	Court	2.3×10^3 to 3.6×10^4	100%
	Binodpur	5.8×10^3 to 2.62×10^4	100%
	Naodapara	1.8×10^3 to 4×10^4	100%
	Saheb Bazar	1.12×10^2 to 1.66×10^3	50%
Fuchka	Laxmipur	2.6×10^4 to 5.1×10^7	100%
	RU Campus	2.8×10^4 to 4.5×10^6	100%
	Court	2.5×10^4 to 3.7×10^5	100%
	Binodpur	3.7×10^4 to 5.0×10^6	100%
	Naodapara	2.7×10^4 to 4.0×10^5	100%
	Saheb Bazar	2.9×10^4 to 6.3×10^7	100%
Chatpoti	Laxmipur	2.1×10^3 to 4.8×10^6	100%
	RU Campus	2×10^3 to 3.7×10^5	100%
	Court	2×10^3 to 3.6×10^4	100%
	Binodpur	1.9×10^3 to 4.1×10^5	100%
	Naodapara	2×10^3 to 3.8×10^4	100%
	Saheb Bazar	1.4×10^3 to 2.3×10^4	100%

Table 4.8. Total *Salmonella* count of RTE food samples on SS agar medium

Food items	Sampling area	Range of cfu/g	Sample contaminated with $\geq 10^1$ cfu/gm
Boiled rice	Laxmipur	1.4×10^1 to 6.8×10^2	84%
	RU Campus	1.1×10^1 to 2.4×10^2	50%
	Court	0	0%
	Binodpur	1×10^2 to 8×10^2	100%
	Naodapara	1.2×10^1 to 5.1×10^2	50%
	Saheb Bazar	0	0%
Vegetable curry	Laxmipur	0.7×10^1 to 2.8×10^2	50%
	RU Campus	0 to 3.2×10^2	84%
	Court	0	0%
	Binodpur	0 to 4.8×10^2	25%
	Naodapara	0	0%
	Saheb Bazar	0 to 2×10^2	17%
Cooked Dal	Laxmipur	8.6×10^2 to 9.2×10^4	100%
	RU Campus	8.4×10^2 to 6.2×10^3	100%
	Court	0 to 6.2×10^2	17%
	Binodpur	6×10^2 to 9×10^3	100%
	Naodapara	0 to 8.2×10^3	17%
	Saheb Bazar	0 to 4×10^2	17%
Fish Curry	Laxmipur	1.5×10^1 to 6.2×10^2	50%
	RU Campus	0 to 1.18×10^2	17%
	Court	0 to 4.2×10^1	0%
	Binodpur	1.3×10^1 to 2×10^2	50%
	Naodapara	0 to 1.2×10^2	17%
	Saheb Bazar	0 to 2×10^1	17%
Chicken curry	Laxmipur	4.1×10^3 to 8.4×10^5	100%
	RU Campus	0 to 4.2×10^2	17%
	Court	0 to 2.2×10^2	34%
	Binodpur	2.1×10^1 to 3×10^2	50%
	Naodapara	3.8×10^1 to 2.8×10^2	50%
	Saheb Bazar	0 to 1.18×10^2	17%
Fuchka	Laxmipur	2.8×10^1 to 5.1×10^3	25%
	RU Campus	2.6×10^1 to 4.3×10^2	25%
	Court	0 to 4.6×10^2	25%
	Binodpur	2.9×10^1 to 5×10^2	50%
	Naodapara	0 to 4.7×10^2	100%
	Saheb Bazar	0 to 3.8×10^2	25%
Chatpoti	Laxmipur	3.3×10^1 to 5.1×10^2	25%
	RU Campus	1.3×10^1 to 2×10^2	25%
	Court	0 to 3×10^2	25%
	Binodpur	1.2×10^1 to 5×10^2	50%
	Naodapara	0 to 5.1×10^2	25%
	Saheb Bazar	0 to 5.2×10^2	25%

Table 4.9. *Staphylococcus* count of RTE food samples on MS agar medium

Food items	Sampling area	Range of cfu/g	Sample contaminated with $\geq 10^6$ cfu/ gm
Boiled rice	Laxmipur	4.2×10^4 to 6.1×10^7	34%
	RU Campus	3.8×10^5 to 4×10^6	34%
	Court	1.4×10^4 to 3.2×10^5	50%
	Binodpur	1.4×10^4 to 2.24×10^5	50%
	Naodapara	3.4×10^4 to 6.2×10^5	50%
	Saheb Bazar	1.2×10^2 to 1.1×10^5	50%
Vegetable curry	Laxmipur	1.44×10^4 to 3.2×10^6	17%
	RU Campus	1.42×10^4 to 4×10^5	34%
	Court	0	0
	Binodpur	1.9×10^4 to 4.2×10^5	50%
	Naodapara	0	0
	Saheb Bazar	1.4×10^3 to 2.8×10^4	50%
Cooked Dal	Laxmipur	4.8×10^5 to 3×10^6	50%
	RU Campus	0.4×10^5 to 2.7×10^6	50%
	Court	1×10^4 to 0.4×10^5	50%
	Binodpur	1×10^5 to 2.2×10^6	50%
	Naodapara	1×10^5 to 1.2×10^6	50%
	Saheb Bazar	2×10^3 to 4.3×10^5	0%
Fish Curry	Laxmipur	2.6×10^4 to 6.4×10^5	0%
	RU Campus	2×10^3 to 5.4×10^4	0%
	Court	8×10^4 to 3×10^5	0%
	Binodpur	1.06×10^4 to 5×10^5	0%
	Naodapara	1.06×10^4 to 5×10^5	0%
	Saheb Bazar	6×10^2 to 2.4×10^4	0%
Chicken curry	Laxmipur	1.2×10^3 to 9.2×10^5	0%
	RU Campus	1.8×10^3 to 7.8×10^4	0%
	Court	2.2×10^3 to 5×10^4	0%
	Binodpur	2×10^3 to 4×10^5	0%
	Naodapara	3×10^2 to 8×10^4	0%
	Saheb Bazar	2×10^2 to 7.4×10^4	0%
Fuchka	Laxmipur	0.2×10^7 to 6×10^8	100%
	RU Campus	6.2×10^6 to 7×10^8	50%
	Court	6.03×10^4 to 8.64×10^6	50%
	Binodpur	1.38×10^5 to 6.2×10^6	75%
	Naodapara	5.2×10^6 to 2.4×10^9	50%
	Saheb Bazar	4.4×10^7 to 7×10^9	100%
Chatpoti	Laxmipur	2×10^7 to 9×10^8	100%
	RU Campus	6.2×10^3 to 8.6×10^6	50%
	Court	3.3×10^4 to 6.3×10^6	50%
	Binodpur	1.03×10^5 to 5×10^7	50%
	Naodapara	8.4×10^4 to 6.0×10^8	75%
	Saheb Bazar	2.5×10^3 to 5.1×10^6	25%

4.2.6. Comparison of mean bacterial load of different RTE food items at different locations

Mean bacterial load of different RTE food items at different locations were analyzed and the result are presented in Fig 4.2-4.8. In case of boiled rice, it was observed that mean bacterial load varied with the bacterial species and sampling sites. The maximum cfu was counted as 12.27 $\log_{10}$ cfu/gm for TVC and followed by 8.96, 7.02 and 4.48 $\log_1$cfu/gm for coliform, *Staphylococcus* and *E. coli*, respectively. The minimum number of bacterial count was recorded for *Salmonella* (0-3.83 $\log_{10}$ cfu/gm). Among the locations, the maximum number of TVC, *Staphylococus, coliforms* and *E. coli* were recorded at Laxmipur and minimum at Shaheb Bazar. But the maximum number of *Salmonella* were recorded at R.U campus and not detected in boiled rice at Court and Shaheb Bazaar areas.The order of mean bacterial count for different types of bacteria in boiled rice were recorded as TVC> coliforms> *Staphylococcus* > *E. coli* > *Salmonella.*

In vegetable curry, it was observed that mean bacterial load varied with the bacterial species and sampmling sites. The maximum mean cfu was counted for TVC (11.46 $\log_{10}$cfu/gm) and followed by 9.98, 6.41 and 5.80 $\log_{10}$cfu/gm for coliforms, *E. coli* and *Staphylococcus,* respectively. The minimum number of bacterial count was recorded for *Salmonella (0-2.2* $\log_{10}$ cfu/gm). Among the locations, the maximum number of TVC, coliforms, *E. coli* and *Staphylococus* were counted at Laxmipur. *Staphylococus* and *Salmonella* were not detected at Court and Naodapara areas. The order of mean bacterial count for different types of bacteria in vegetable curry were recorded as TVC > coliforms > *E. coli* > *Staphylococcus* > *Salmonella.*

In chicken curry, it was observed that mean bacterial load varied among the bacterial types and sampling sites. The maximum mean cfu was counted for TVC (12.64 $\log_{10}$ cfu/gm) and followed by 10.94, 5.41 and 5.24 $\log_{10}$cfu/gm for coliforms, *Staphylococcus* and *E. coli* respectively. The minimum bacterial count was recorded for *Salmonella* (1.29 - 5.17 $\log_{10}$ cfu/gm). Among the locations, the maximum number of TVC, coliforms, *Staphylococcus, E. coli* and *Salmonella* were recorded at Laxmipur and minimum at Shaheb bazaar. The order of mean bacterial count for different types of bacteria in vegetable curry were recorded as TVC > coliforms > *Staphylococcus* > *E. coli* > *Salmonella.*

In fish curry, it was observed that mean bacterial load varied among the bacterial types and sampling sites. The maximum mean of cfu was counted for TVC (12.32 $\log_{10}$ cfu/gm) and followed by 10.94, 5.30 and 4.36 $\log_{10}$cfu/gm for coliform, *staphylococcus* and *E. coli* respectively. The minimum bacterial number was recorded for *Salmonalla* (0.52-3.36 $\log_{10}$cfu/gm). Among the sampling sites, the maximum number of TVC, coliforms, *E. coli*, *Salmonella* were recorded at Laxmipur and *Staphylococus* at Naodapara. But the minimum was recorded at Shaheb Bazaar. The order of mean bacterial count for different types of bacteria in vegetable curry were recorded as TVC > coliforms > *Staphylococcus* > *E. coli* > *Salmonella.*

In cooked dal mean bacterial load varied with the bacterial types and sampling sites. The maximum mean cfu was counted for TVC (12.48 $\log_{10}$ cfu/gm) and followed by 10.26, 6.36 and 6.22 $\log_{10}$cfu/gm for coliform, *E. coli* and *Staphylococcus,* respectively. The minimum number of bacterial count was recorded for *Salmonella* (1.83-4.17 $\log_{10}$cfu/gm). Among the sampling sites, the maximum TVC, coliform, *E. coli*, *Salmonella* an

Staphylococcus were counted at Laxmipur and the minimum was recorded at Shaheb bazar. The order of mean bacterial count for different types of bacteria in vegetable curry were recorded as TVC > coliform > *Staphylococcus* > *E. coli* > *Salmonella.*

In chatpoti, it was observed that mean bacterial load varied with the bacterial types and sampling sites. The maximum mean cfu was counted for TVC (11.11$\log_{10}$cfu/gm) and followed by 8.54, 8.15 and 6.35 $\log_{10}$cfu/gm for S*taphylocus,* coliform and *E. coli,* respectively. The minimum number of bacterial count was recorded for *Salmonella* (1.11 - 2.41 $\log_{10}$cfu/gm). Among the sampling sites, the maximum TVC, *E. coli, Salmonella* and *Staphylococcus* was recorded at Laxmipur and *coliform* was recorded at R.U campus. But the minimum was recorded at court area. The order of mean bacterial count for different types of bacteria in chatpoti were recordted as TVC>*Staphylococcus*>*Coliform*>*E .coli* > *Salmonella.*

In fuchka, it was observed that mean bacterial load varied with the bacterial types and sampling sites. The maximum mean cfu was counted for TVC (13.12 $\log_{110}$cfu/gm) and followed by 8.68, 8.53 and 7.23 13.12 $\log_{110}$cfu/gm for coliform, *Staphylococcus* and *E. coli* respectively. The minimum number of bacterial count was recorded for *Salmonella* (1.11-1.41 $\log_{10}$cfu/gm). Among the sampling sites, the maximum TVC, coliform *and E. coli* were recorded at Shaheb bazaar area and minimum was recorded at Court and Laxmipur areas. But the maximum number of *Salmonella* sp. was recorded at Laxmipur and minimum at Court areas. For *Staphylococus* maximum number was recorded at R.U campus and at Court area. The order of mean bacterial count for different types of bacteria in fuchka were recorded as TVC > *coliform* [illegible] *phyloc* [illegible] *E. coli* > *Salmonella.*

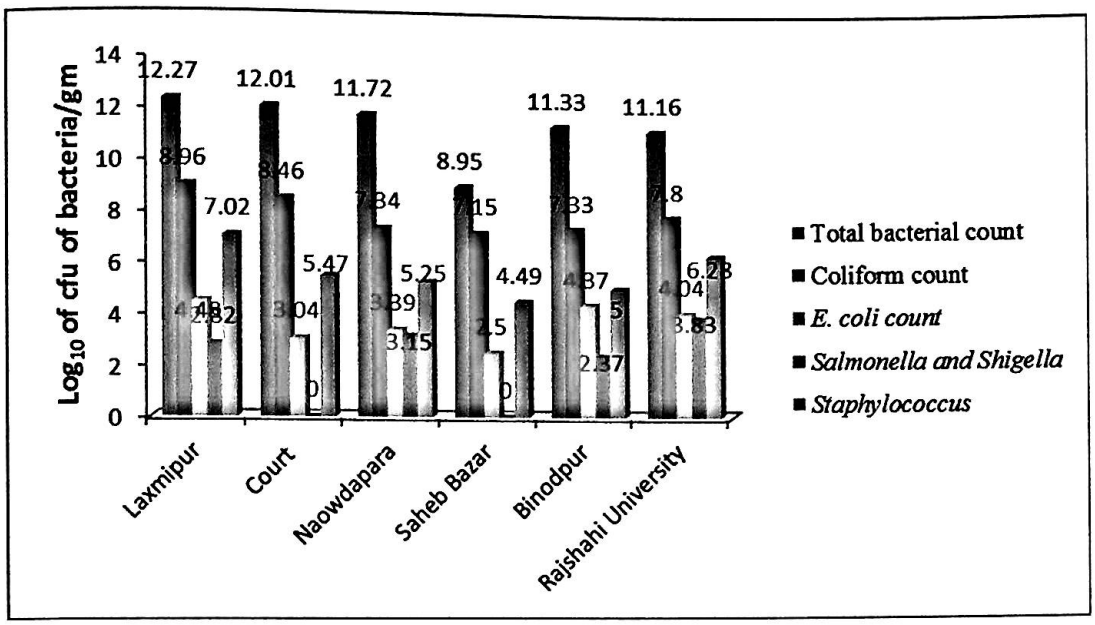

Fig 4.2. Mean bacterial load of boiled rice at different locations

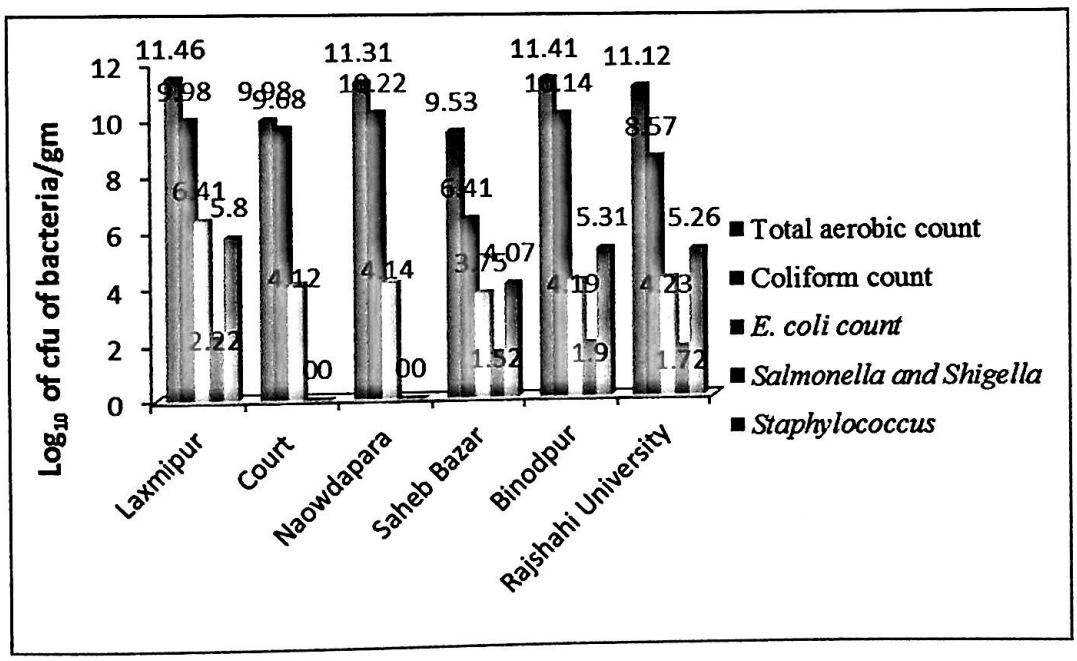

Fig 4.3. Mean bacterial load of vegetable curry at different locations

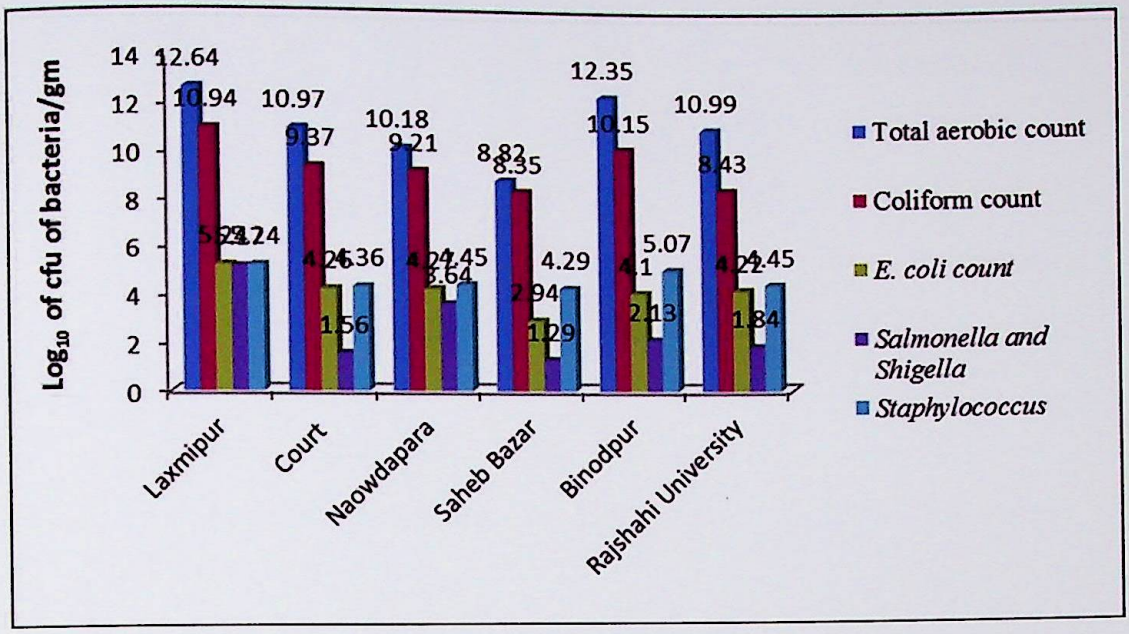

Fig 4.4. Mean bacterial load of chicken curry at different locations

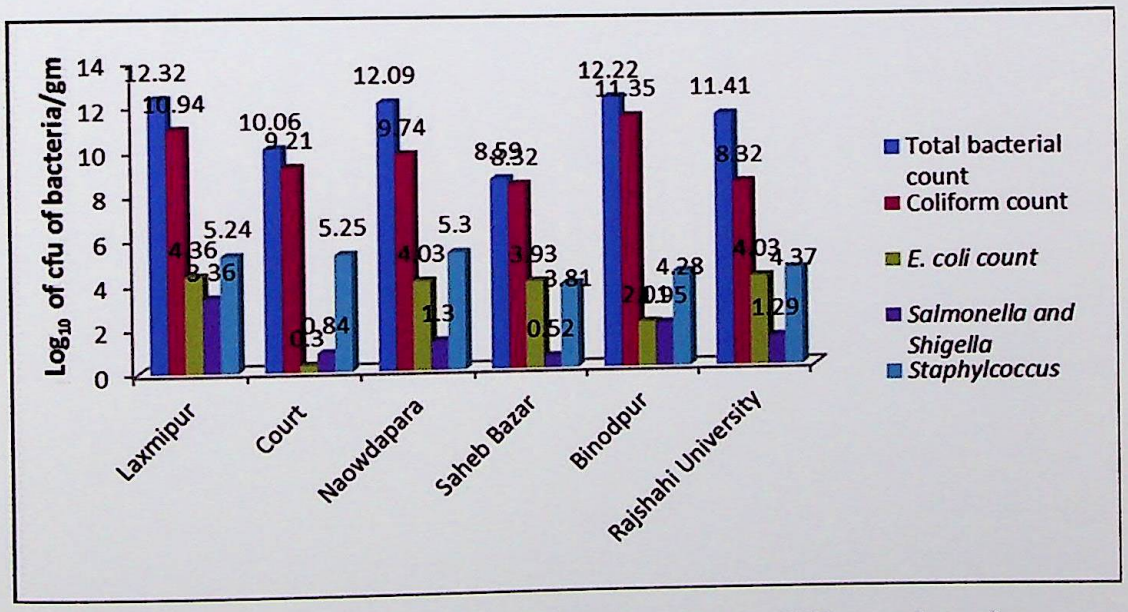

Fig 4.5. Mean bacterial load of fish curry at different locations

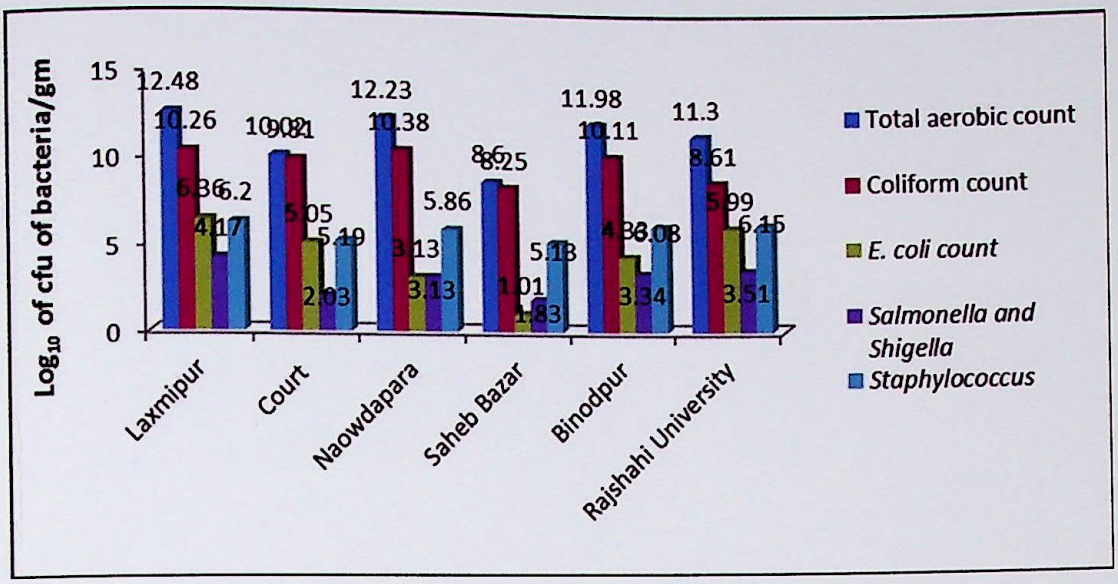

Fig 4.6 Mean bacterial load of cooked dal at different locations

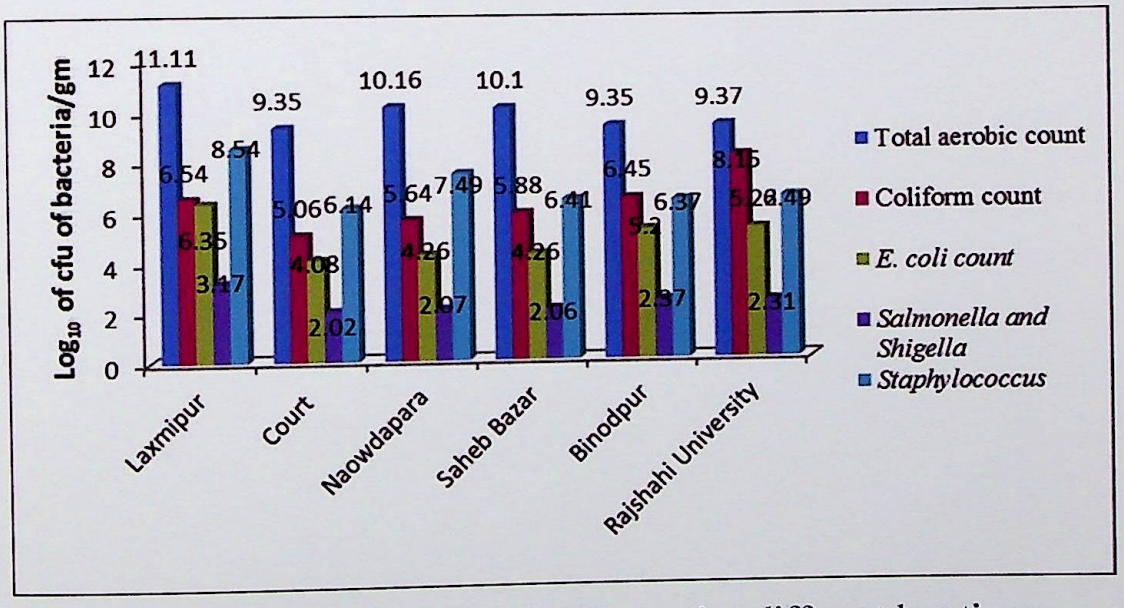

Fig 4.7 Mean bacterial load of chatpoti at different locations

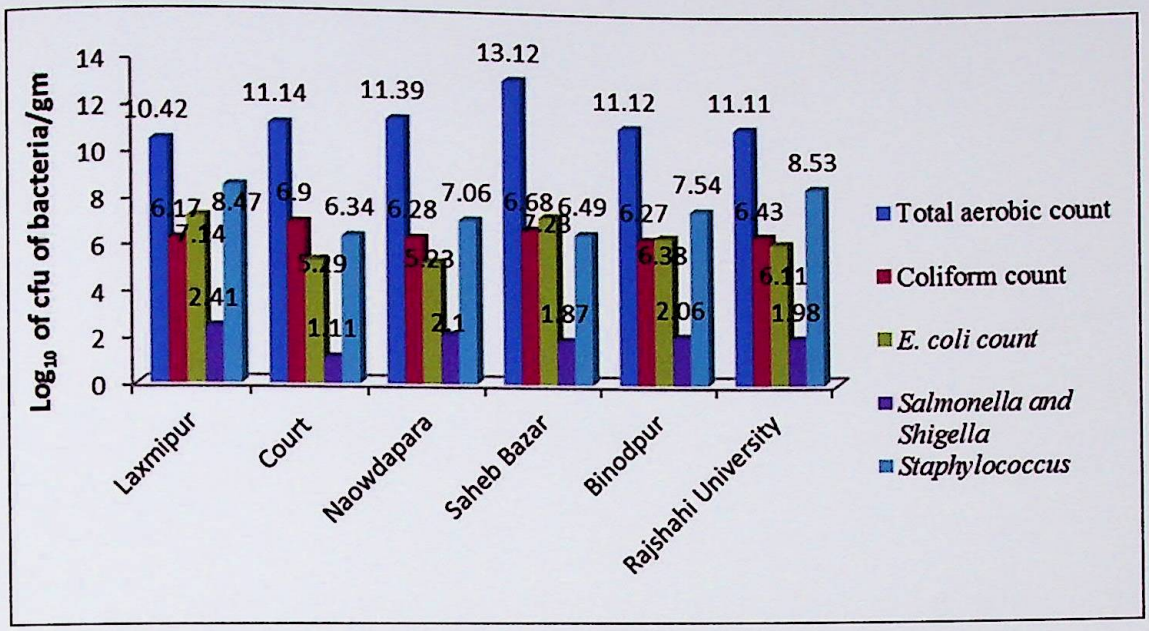

Fig 4.8. Mean bacterial load of fuchka at different locations

4.3 Isolation and Identification of the bacterial isolates of different RTE foods

4.3.1 Isolation of bacterial isolates from RTE foods

For isolation of bacterial isolates, seven types of RTE food samples were plated on five media i.e. NA agar, MacConkey agar, EMB agar , SS agar and MS agar medium. After 24 hours, bacterial colonies were visible on the surface of NA, MacConkey, EMB, SS and MS agar plates and the morphologically distinct colonies were identification and coded with their sources (Table 4.2.2).

4.3.2 Characterizations of the isolates on some nutrient media

The colony morphology of the isolates on nutrient agar, MacConkey agar, EMB agar and SS agar were observed according to form, color, elevation, margin, and surface and the results are presented in (Table 4.6 - 4.10).

Table 4.10. Cultural characteristics of the isolates on NA medium

Group code & total number of isolates	Form	Color	Elevation	Margin	Surface
Gr1(R)-20 isolates, Gr3(D)-3 isolates	Round	White	Flat	Lobate	Smooth
Gr1(D)-35 isolates, Gr2(D)-29 isolates, Gr3(V)-48 isolates, Gr7(V)-12 isolates	Round	White	Flat, Raised	Lobate	Rough
Gr1(V)-29 isolates, Gr6(C)-28 isolates, Gr9(F)-18 isolates,Gr1(Ch)-48 isolates,Gr2(Fu)-40 isolates,Gr1(v)-29 isolates	Round, Regular	Off white	Flat, Raised	Entire	Rough

[Representative isolates in each group are shown in Table 4.16]

Table 4.11. Cultural characteristics of the isolated bacteria on MacConkey medium

Group code & number of isolates	Form	Coluor	Elevation	Margin	Surface
Gr3(R)-16 isolates ,Gr2(R)-38 isolates	Round, Irregular	Pink	Raised	Entire	Smooth
Gr1(F)-46 isolates ,Gr4(D)-9 isolates ,Gr9(V)-4 isolates ,Gr3(V)-38 isolates ,Gr8(V)-11 isolates , Gr4(C)-24 isolates	Round, Regular	Pink	Flat, Raised	Lobate	Smooth
Gr2(F)-1 isolates,Gr1(C)-16 isolates, Gr2(C)-16 isolates Gr2(Ch)-22 isolates ,Gr2(D)-22 isolates	Round	Light pink	Raised	Entire	Rough

[Representative isolates in each group are shown in Table 4.16]

Table 4.12. Cultural characteristics of the isolates on EMB medium

Group code & number of isolates	Form	Coluor	Elevation	Margin	Surface
Gr4(R)-12 isolates ,Gr6(R)-30 isolates	Round	Deep purple	Raised	Wavy	Smooth
Cr5(R)-2 isolates, Gr7(R)-12 isolates ,Gr6(D)-16 isolates, Gr3(F)-8 isolates ,Gr7(F)-22 isolates ,Gr3(Fu)-18 isolates ,Gr4(Ch)-8 isolates	Round	Purple	Raised	Wavy	Rough
Gr3(C)-18 isolates ,Gr5(D)-14 isolates ,Gr6(D)-16 isolates	Round	Light purple	Raised	Entire	Rough
Gr7(F)-22 isolates ,Gr1(Fu)-4 isolates ,Gr3(Ch)-12 isolates	Round, Regular	Off White	Flat	Lobate	Smooth

[Representative isolates in each group are shown in Table 4.16]

Table 4.13. Cultural characteristics of the isolates on SS Medium

Group code & number of isolates	Form	Coluor	Elevation	Margin	Surface
Gr5(C)-20 isolates , Gr8(D)-32 isolates , Gr5(V)-12 isolates	Round, Regular	Gray	Raised	Elongated	Smooth
Gr8(R)-12 isolates ,Gr2(D)-29 isolates,Gr5(F)-18 isolates	Round	Gray	Flat	Entire	Smooth
Gr1(F)-46 isolates,Gr5(F)-18 isolates	Round, Regular	Gray	Flat	Lobate	Rough
Gr4(C)-24 isolates,Gr4(Fu)-18 isolates	Round, Irregular	Violate	Raised	Elongated	Smooth

[Representative isolates in each group are shown in Table 4.16]

Table 4.14. Cultural characteristics of the isolates on MS Medium

Group code & number of isolates	Form	Colour	Elevation	Margin	Surface
Gr9(R)-28 isolates , Gr8(D)-32 isolates , Gr6(V)-14 isolates , Gr8(F)-12 isolates , Gr4(C)-24 isolates , Gr6(Fu)-20 isolates , Gr6(Ch)-16 isolates	Irregular	White cream,pink red,yellow orange	Flat	Lobate	Rough

[Representative isolates in each group are shown in Table 4.16]

4.3.3. Microscopic features of the isolates

The microscopic features of the isolates are presented in Table4.11, and plate 4.3. After Gram staining bacterial cells were observed under microscope and their characteristics were recorded. In this investigation, total 1140 isolates were isolated. According to Gram staining results the isolates were Gram negative and Gram positive (4.12) and varied their shape as long rod, rod shape and short rod shape and coccus.

4.3.4 Motility test result

The growth of motile and non-motile bacteria occurs in the stab culture. In this study out of 1140 isolates 861 isolate showed positive result for motility test and rest of the isolates showed negative result for motility test. (Table 4.12 and Plate 4.5 A).

4.3.5 Catalase test result

Among the 1140 isolates, 1003 isolates showed positive catalase test result because it liberated gas (O_2) from hydrogen peroxide (H_2O_2) by enzymatic degradation (Table 4.12 - 4.18 and Plate 4.5D) and rest of the isolates showed negative results for catalase test

Table 4.15: Microscopic features of the isolated bacteria

Group code and number of isolates	Shape of vegetative cells
Gr6(R)-30 isolates, Gr2(R)-34 isolates, Gr4(R)-12 isolates, Gr1(R)-20 isolates, Gr5(R)-2 isolates, Gr3(D)-3 isolates, Gr2(D)-29 isolates, Gr2(D)-29 isolates, Gr2(D)-24 isolates, Gr7(V)-12 isolates, Gr3(V)-48 isolates, Gr8(V)-11 isolates Gr1(F)-22 isolates, Gr2(F)-12 isolates, Gr4(C)-24 isolates Gr5(Fu)-14 isolates, Gr4(Ch)-8 isolates, Gr2(Ch)-28 isolates	Long rod
Gr8(R)-12 isolates, Gr3(R)-16 isolates, Gr1(D)-35 isolates ,Gr4(D)-9 isolates, Gr8(D)-32 isolates, Gr9(V)-4 isolates ,Gr2(V)-16 isolates, Gr1(V)-29 isolates, Gr4(F)-22 isolates, Gr9(F)-18 isolates, Gr3(F)-8 isolates, Gr7(F)-22 isolates, Gr5(F)-18 isolates, Gr6(F)-2 isolates, Gr6(C)-28 isolates, Gr1(C)-16 isolates, Gr4(C)-24 isolates, Gr7(C)-10 isolates, Gr4(Fu)-18 isolates, Gr2(Fu)-30 isolates, Gr1(Fu)-4 isolates, Gr3(Fu)-18 isolates, Gr1(Ch)-48, isolates, Gr2(Ch)-22 isolates, Gr4(Ch)-12 isolates, Gr5(V)-12 isolates	Rod shape
Gr7(R)-12 isolates, Gr5(D)-14 isolates, Gr6(C)-16 isolates, Gr3(V)-38 isolates, Gr1(V)-29 isolates, Gr4(V)-13 isolates, Gr9(F)-18 isolates, Gr3(F)-58 isolates, Gr5(C)-20 isolates, Gr3(C)-18 isolates, Gr2(C)-16 isolates	Short rod
Gr9(R)-28 isolates, Gr6(V)-14 isolates, Gr8(C)-14 isolates, Gr9(F)-12 isolates, Gr8(D)-32 isolates, Gr6(Fu)-20 isolates, Gr6(Ch)-6 isolates	Coccus

[Representative isolates in each group are shown in Table 4.16]

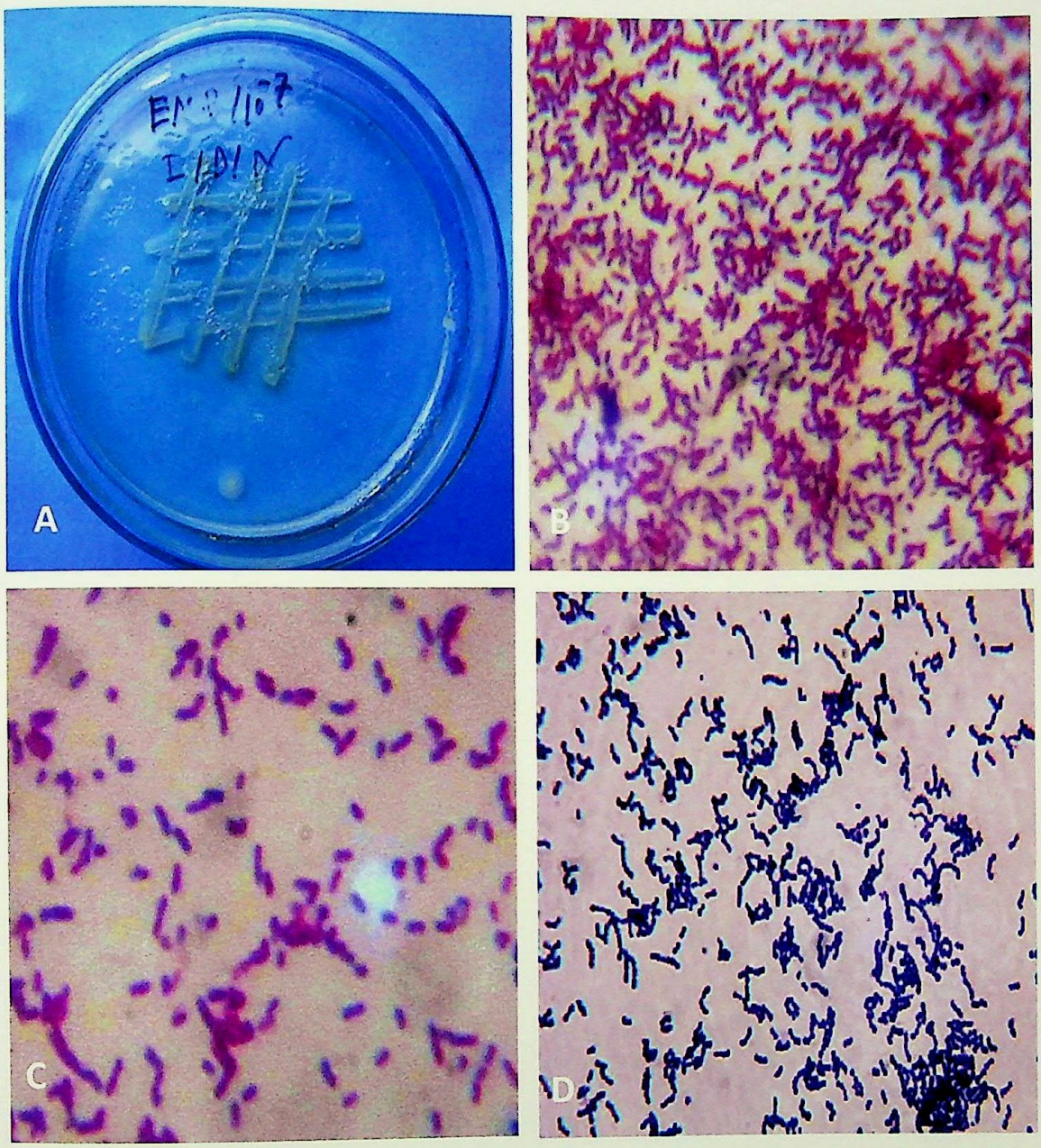

Plate 4.5. Photographs showing bacterial pure culture (A); Gram Negative bacteria (B-C) and Gram positive bacteria (D).

Table 4.16 Gram staining, motality and catalase test results

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
1	**Gr6(R)-30:** LCRS-31,LCRS-32,RTRS-71,LTRS-72,SBRE-621, SBRS-622,SARE-701,SARE-702,RSRE-181,RSRE-182,RKRE-221,RKRE-222,BRRN-361,BRRN-362,CMRM-251,CMRM-252,CBRM-331,CBRM-332,CBRE-341,CBRE-342,CKRE-301, CKRE-302,NTRM-491,NTDM-492,BARM-411,BARM-412,	-	+	+
2	**Gr8(R)-12:** SMRS-671,SMRS-672,RKRS-231,RKRS-232, BRRS-391,BRRS-392,BARS-431, BARS-432,BHRS-471, BHRS-472,CBRS-351, CBRS-352	-	+	+
3	**Gr3(R)-16:** LCRM-11,LCRM-12,LTRM-51,LTRM-52,LERM-91, LERM-92,SMRM-651, SMRM-652,SARM-691,SARM-692, BRRM-371,BRRM-372,CKRM-291,CKRM-292,NMRM-531, NMRM-532	-	+	+
4	**Gr2(R)-38:** NTRN-481,NTRN-482,NERM-561,NERM-562, LERS-111,LERS-112,RCRN-121,RCRN-122,RKRN-201, RKRN-202,RCRE-141,RCRE-142,RCRS-191,RCRS-192, RCRM-131,RCRM-132,RKRM-211,RKRM-212,CMRS-271, CMRS-272, SBRN-601,SBRN-602,SARN-681,SARN-682, SMRN-641,SMRN-642,BBRM-611,BBRM-612,NTRS-511, NTRS-512,NMRS-551,NMRS-552, SARS-711,SARS-712,	-	+	+
5	**Gr4(R)-12:** LCRE-21,LCRE-22,LTRE-61,LTRE-62,RSRM-171, RSRM-172,BHRE-461, BHRE-462,CKRS-311,CKRS-312, RERE-581,RERE-582	-	+	+
6	**Gr1(R) 20:** LCRN-1,LCRN-2,LERN-81,LERN-82,LTRN-41, LTRN-42,RSRN-161,RSRN-162,BARN-401,BARN-402, BHRN-441,BHRN-442,CMRN-241,CMRN-242, CKRN-281, CKRN-282,CBRN-321, CBRN-322,NMRN-521,NMRM-522	-	+	+
7	**Gr5(R)-2:** LERE-101,LERE-102	-	+	+
8	**Gr7(R)-12:** SMRE-661,SMRE-662,BRRE-381,BRRE-382, BARE-421,BARE-422,CMRE-261, CMRE-262,NTRE-501, NTRE-502,NMRS-541,NMRS-542	-	+	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
9	**Gr9(R)-28:** LCRMn-913,LCRMn-914,LTRMn-923,LTRMn-924,LER-933,LERMn-934, SBRMn-1033,SBRMn-1034, RCRMn-943, RCRMn-944, RSRMn-953, RSRMn-954, RKRMn-963, RKRMn-964, BHRMn-1083, BHRMn-1084, BARMn-1073, BARMn-1074, CMRMn-973, CMRMn-974, CKRMn-983, CKRMn-984, CBCMn-993, CBCMn-994, NTRMn-1003, NTRMn-1004, NERMn-1023, NERMn-1024	+	–	+
10	**Gr1(D)-35:** LCDN-9,LCDN-10,BRDM-379,BADM-419,BADM-420,CKDM-299,CKDM-300,CBDM-339,CBDM-340,RCDN-169, RCDN-170,RKDN-209,RKDN-210, NTDN-489,NTDN-490,NMDM-529,NMDN-530,BADN-410,CMDM-259,CMDM-260LTDM-59,LTDM-60,SADM-699,SADM-700,BRDM-380, CMDN-249,CMDN-250,CKDN-289,CKDN-290,CBDN-329, CBDN-330,RSRM-179, RSDM-180	-	+	+
11	**Gr3(D)-3:** BADN-409,RSDN-129,SBDN-609	-	-	-
12	**Gr5(D)-14:** SBDE-629,SBDE-630,SMDE-659,SBDE-660, BADE-429,BADE-430,CKDE-309, CKDE-310,CBDE-349, CBDE-350, RCDE-149,RCDE-150,RSDE-189,RSDE-190	-	-	+
13	**Gr2(D)-29:** LTDN-49,LTDN-50,LCDM-19,LCDM-20,LEDE-109,LEDE-110,LTDS-79,LTDS-80,SBDM-620,BRDN-369, BRDN-370,BHDN-449,BHDN-450,BHDM-459, BHDM-460, BRDS-399,BRDS-400,RKDM-219,RKDM-220,RCDM-139, RCDM-140,NEDM-579,NEDM-580, NEDE-589, NEDE-590,NEDS-599, CMDS-279	-	+	+
14	**Gr2(D)-22:** LTDN-49,LTDN-50,LCDM-19,LCDM-20,LEDE-109,LEDE-110,LTDS-79,LTDS-80,SBDM-620,BRDN-369, BRDN-370,BHDN-449,BHDN-450,BHDM-459. BHDM-460, BRDS-399,BRDS-400,RKDM-219,RKDM-220,RCDM-139, RCDM-140,NEDM-579	-	+	+
15	**Gr2(D)-29:** LTDN-49,LTDN-50,LCDM-19,LCDM-20,LEDE-109,LEDE-110,LTDS-79,LTDS-80,SBDM-620,BRDN-369, BRDN-370,BHDN-449,BHDN-450,BHDM-459, BHDM-460, BRDS-399,BRDS-400,RKDM-219,RKDM-220,RCDM-139, RCDM-140,NEDM-579,NEDM-580, NEDE-589, NEDE-590,NEDS-599, CMDS-279	-	+	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
16	**Gr6(D)-16:** LCDE-29,LCDE-30,LTDE-69,LTDE-70,SADE-709, SADE-710,BRDE-381, BRDE-382,BARE-421,BARE-422, CMDE-229,CMDE-270,RKDE-229,RKDE-230,NTDE-509, NTDE-510, NMDE-549,NMDE-550	-	+	+
17	**Gr8(D)-32:** LCDS-39,LCDS-40,LEDS-119,LEDS-120,LCDS-39, LCDS-40,LEDS-119,LEDS-120,SBDS-639,SBDS-640,SMDS-679,SMDS-680,SADS-719,SADS-720,BADS-439,BADS-440, BHDS-479,BHDS-480,CKDS-319,CKDS-320,CBDS-359, CBDS-360,RCDS-159,RCDS-160,RSDS-199,RSDS-200, RKDS-239,RKDS-240,NTDS-519,NTDS-520,NMDS-559, NMDS-560	+	+	+
18	**Gr4(D)-9:** LEDM-99,LEDM-100,SBDM-619,SMDM-659, SMDM-660,NTDM-499,NTDM-500,NMDM-539,NMDM-540	-	+	+
19	**Gr8(D)-32:** LTDMn-921,LCDMn-922,LTDMn-931,LTDMn-932,LEDMn-941,LEDMn-942, RSDMn-961,RSDMn-962, RCDMn-951,RCDMn-952,RKDMn-971,RKDMn-972,NTDMn-1011,NTDMn-1012,NMDMn-1021NMDMn-1022,CMDMn-981, CMDMn-982,CKDMn-991,CKDMn-992,CBDMn-1001, CBDMn-1002,BRDMn-1063,BRDMn-1064,BADMn-1081, BADMn-1082,SBDMn-1041,SBDMn-1042, SMDMn-1061, SMDMn-1062,SADMn-1071,SADMn-1072	-	+	+
20	**Gr9(V)-4:** CKVM-295,CKVM-296,NTVE-505,NTVE-506	-	+	+
21	**Gr3(V)-48:** LCVM-55,LCVM-56,SDVM-615,SBVM-616,BHVM-455,BRVS-395,BRVS-396, BAVS-435,BAVS-436, BHVS-475,BHVS-476,CMVE-265,CKVE-305,CMVS-275, CMVS-276,CKVS-316,RKVE-225,RKVE-226,SAVN-685,SAVN-686,SMVS-675, SMVS-676,SAVS-715,SAVS-716,BHVN-445,BHVN-446,CMVN-245,CMVN-246,CBVN-325, CBVN-326,CKVN-285,CKVN-286,RSVS-165, RSVS-166, RKVM-215,RKVM-216,RCVM-137,RCVM-138,BRVE-385, BRVE-386,BHVE-465, BHVE-466,NMVM-536, RKVS-235,RKVS-236,LTVS-76,NEVE-585,SAVE-705	-	+	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
22	**Gr1(V)-29:** LCVN-5,LCVN-6,LEVN-85,LEVN-86,LTVN-45, LTVN-46,LEVS-115,LEVS-116,LTVS-75,LTVS-76,SBVE-625,SBVE-626,SMVE-665,SBVS-635,SBVS-636, CMVM-255,CMVM-256,CBVM-335,CBVM-336,CMVE-266, CKVE-306	-	+	+
23	**Gr2(V)-16:** LCVM-15,LCVM-16,LEVM-95,LEVM-96,LCVE-25.LCVE-26,LTVE-65,LTVE-66,BAVE-425,BAVE-426, RCVN-125,RCVN-126,RKVN-205,RKVN-206,NEVN-565, NEVN-566,BHVM-456	-	-	+
24	**Gr1(V)-29:** Gr1(V)-30,LCVN-5,LCVN-6,LEVN-85,LEVN-86, LTVN-45,LTVN-46,LEVS-115,LEVS-116,LTVS-75,LTVS-76, SBVE-625.SBVE-626,SMVE-665,SBVS-635, SBVS-636,CMVM-255,CMVM-256,CBVM-335,CBVM-336, CMVE-266,CKVE-306	-	+	+
25	**Gr7(V)-12:** SBVN-605,SBVN-606,SMVN-645,SMVN-646, BRVN-365,BRVN-566,BAVN-405,BAVN-406,NTVN-485, NTVN-486, NMVN-525,NMVN-526	-	+	+
26	**Gr3(V)-48:** LCVM-55,LCVM-56,SDVM-615,SBVM-616,BHVM-455,BRVS-395,BRVS-396, BAVS-435,BAVS-436, BHVS-475,BHVS-476,CMVE-265,CKVE-305,CMVS-275, CMVS-276,CKVS-316,RKVE-225,RKVE-226,SAVN-685,SAVN-686,SMVS-675, SMVS-676,SAVS-715,SAVS-716,BHVN-445,BHVN-446,CMVN-245,CMVN-246,CBVN-325, CBVN-326,CKVN-285,CKVN-286,RSVS-165, RSVS-166, RKVM-215,RKVM-216,RCVM-137,RCVM-138,BRVE-385, BRVE-386,BHVE-465, BHVE-466,NMVM-536, RKVS-235,RKVS-236,LTVS-76,NEVE-585,SAVE-705	-	+	+
27	**Gr6(V)-14:** LCVMn-917,LCVMn-918,SAVMn-1057,SAVMn-1058,BAVMn-1087,BAVMn-1088,CBVMn-997,CBVMn-998, RCVMn-947,RCVMn-948,RKVMn-967, RKVMn-968,NTVMn-1007,NTVMn-1008	+	-	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
28	**Gr8(V)-11:** SMVN-655,SMVM-656,SAVM-695,SAVM-696,BRVM-376,BAVM-415,BAVM-416,NTVM-495, NTVM-496, NMVM-535,NMVM-436	-	+	+
29	**Gr4(V)-13:** LEVE-105,LEVE-106,SAVE-706,SAVE-706, CBVE-345,RCVE-145,RCVE-146, RSVE-185,RSVE-186, NMVE-545,NEVE-585,BRVE-385,BHVE-465	-	+	+
30	**Gr5(V)-12:** LCVS-35,LCVS-36,CKVS-315,CBVS-355,CBVS-356,RSVS-196,NTVS-515, NTVS-516,NMVS-555,NMVS-556, NEVS-595,NEVS-596	-	-	-
31	**Gr1(F)-22:** LCFN-7,LCFN-8,LTFN-14,LTFN-15,LEFN-87, LEFN-88,SMFM-657,SMFM-65, SAFM-997,SAFM-998, BRFM-377,BRFM-378,BAFM-417,BAFM-418,BAFS-437, BAFS-438,CMFN-247,CMFN-248,CKFN-287,CKFN-288, CBFN-327, CBFN-328	-	+	+
32	**Gr4(F)-22:** LCFE-27,LCFE-28,LTFE-67,,LTFE-68,SBFN-607, SBFN-608,SBFS-337,SBFS-338,CMFM-257,CMFM-258, CMFE-267,CMFE-268,NEFN-567,NEFN-568, NTFM-497, NTFM-498,NMFM-537,NMFM-538,NTFE-507,NTFE-508, NMFE-547,NMFE-548	-	-	-
33	**Gr1(F)-22:** LCFN-7,LCFN-8,LTFN-14,LTFN-15,LEFN-87, LEFN-88,SMFM-657,SMFM-65, SAFM-997,SAFM-998, BRFM-377,BRFM-378,BAFM-417,BAFM-418,BAFS-437, BAFS-438,CMFN-247,CMFN-248,CKFN-287,CKFN-288, CBFN-327, CBFN-328	-	+	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
34	**Gr9(F)-18:** SMFN,-647SMFN,-648,SAFN-687,SAFN-688, BHFN-447,BHFN-448,RCFN-127, RCFN-128,RKFN-207, RKFN-208,RCFM-135,RCFM-136,RKFE-227,RKFE-228, RCFMn-917,RCFMn-918, RKFMn-967, RKFMn-968.	-	+	+
35	**Gr1(F)-48:** LCFN-7,LCFN-8,LTFN-14,LTFN-15,LEFN-87, LEFN-88,SMFM-657,SMFM-65,SAFM-997,SAFM-998, BRFM-377,BRFM-378,BAFM-417,BAFM-418,BAFS-437, BAFS-438,CMFN-247,CMFN-248,CKFN-287,,CKFN-288, CBFN-327, CBFN-328,CBFM-337,CBFM-338,NTFN-487, NTFN-488,NMFN-527,NMFN-528,NMFS-557, NEFS-597,NEFS-598,SMFN-647,SMFN-648, SAFN-687,SAFN-688,LEFS-117,LEFS-118,SBFE-627,SBFE-628,BAFN-407,BAFN-408,CBFS-357,CBFS-358,RSFN-167, RSFN-168,RCFS-157,RCFS-158,RSFS-197,RSFS-198	-	+	+
36	**Gr3(F)-8:** LEFM-97,LEFM-98,SBFM-617,SBFM-618,BHFM-457, BHFM-458,CKFM-297, CKFM-298	-	+	+
37	**Gr8(F)-12:** LTFMn-929,LTFMn-930,SAFMn-1059,SAFMn-1060,BRFMn-1069,BRFMn-1070,CMFMn-979,CMFMn-980, CKFMn-989, CKFMn-990,NMFMn-1019, NMFMn-1020	+	-	+
38	**Gr2(F)-12** LCFM-17,LCFM-17,LTFM-57,LTFM-58,BRFN-367, BRFN-368,RKFM-217, RKFM-218,RSFM-177,RSFM-178, NEFM-577,NEFM-588	-	+	+
39	**Gr7(F)-22:** LEFE-107,LEFE-108,SAFM-667,SAFM-668,SAFE-707,SAFE-708,BRFE-387, BRFE-388,BAFE-427,BAFE-428, BHFE-467,BHFE-468,CKFE-307,CKFE-308, CBFE-347,CBFE-348,RCFE-147,RCFE-148,RSFE-187,,RSFE-188,NEFE-587, NEFE-588.	-	+	+
	Gr6(F)-2: RKFS-237, RKFS-238.	-	+	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
41	**Gr5(F)-20:** LCFS-37,LCFS-38,LTFS-77,LTFS-78,SMFS-677, SMFS-678,SAFS-717,SAFS-718, BRFS-397,BRFS-398,BHFS-477,BHFS-478,CMFS-277,CMFS-278,CKFS-317,CKFS-318, NTFS-517,NTFS-518,SMFN-647,SAFN-687	-	-	+
42	**Gr6(C)-19:** LTCS-73,LTCS-74,CBCN-323,CBCN-324,SACE-703,SACE-704,BHCN-443, BHCN-444,BRCS-391,BRCS-394, CMCN-243,CMCN-244,CKCN-283,CKCN-284, NECS-593,NECS-594		+	+
43	**Gr1(C)-16:** LCCN-3,LCCN-4,LCCM-13,LCCM-14,LTCM-53, LTCM-54,BRCM-373, CMCM-253,CMCM-254, CBCM-333, RKCM-213,RKCM-214, RSCM-173, RSCM-174, NECM-573,NECM-574.	-	+	+
44	**Gr4(C)-24:** LCCS-33,LCCS-34,LECS-113,LECS-114,CKCS-313,CKCS-314,RCCN-123, RCCN-124,SBCS-633,SBCS-634, BACS-433,BACS-434,BHCS-473,BHCS-474, CMCS-273, CMCS-274,CBCS-353,CBCS-354,RSCS-193,RSCS-194,NTCS-513, NTCS-514,NMCS-553,NMCS-554	-	+	+
45	**Gr3(C)-18:** LCCE-23,LCCE-24,LTCE-63,,LTCE-64,LECE-103, LECE-104,SBCE-623,SBCE-624,SMCE-663,SMCE-664, BHCE-463,BHCE-464,CMCE-263,,CMCE-264, CKCE-303, CKCE-304,RKCE-223,RKCE-224	-	+	+
46	**Gr4(C)-24:** LTCN-43,LTCN-44,LECN-83,LECN-84,SACN-683, SACN-684,SBCM-613, SBCM-614,SMCM-653,SMCM-654, BHCM-454,CKCM-293,CKCM-294, RCCM-133,RCCM-134,RCCE-143,RCCE-144,RSRE-183, RCCS-153,RCCS-154, NMCN-524, NECN-563, NECN-564,	-	+	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
47	**Gr4(C)-4:** NTCM-493, NTCM-494, NMCM-533, NMCM-534	-	+	+
48	**Gr8(C)-16:** LCCMn-915,LCCMn-915,SBCMn-1035,SBCMn-1036,BACMn-1075,BACMn-1076,BHCMn-1085,BHCMn-1086,CMCMn-973,CMCMn-974,CKCMn-983, CKCMn-984, RCCMn-943,RCCMn-944,NTCMn-1033,NTCMn-1034	+	-	+
49	**Gr2(C)-16:** LECM-93,LECM-94,SACM-693,SACM-694,BRCN-363,BRCN-364,BACN-403, BRCE-383, BACE-423, RSCN-163,RSCN-164,RKCN-203, NTCN-483,NTCN-484,NTCE-503, NTCE-504	-	+	+
50	**Gr7(C)-10:** SMSS-673,SMSS-674,SACS-713,SACS-714,CBCE-343, CBCE-344,BRCN-363, BRCN-364,SMCN-343,SMCN-644	-	-	-
51	**Gr4(Fu)-18:** LFuSS-733,LFuSS-734,NFuSS-895,NFuSS-896, SFuSS-857,SFuSS-858,SFuSS-863,SFuSS-864,BFuSS-797, BFuSS-798,RFuES-829,RFuES-830,NFuNS-883,NFuNS-884, SFuNS-851, SFuNS-852,RFuNS-817,RFuNS-818	-	-	+
52	**Gr2(Fu)-40:** LFuNS-721,LFuNS-722,LFuNS-723,LFuNS-724, CFuNS-753,CFuNS-754,CFuNS-755,CFuNS-756,NFuMS-885, NFuMS-886,SFuNS-849,SFuNS-850,BFuNS-785, BFuNS-786, BFuNS-787,BFuNS-788,BFuMS-791,BFuMS-792,RFuNS-819, RFuFS-820,LFuMS-725,LFuMS-726,LFuMS-727,LFuMS-728, CFuMS-757, CFuMS-758,CFuMS-759, NFuNS-881, NFuNS-882,NFuMS-887, NFuMS-888, SFuMS-853,SFuMS-854, SFuMS-855,SFuMS-856,BFuMS-789, BFuMS-790, RFuMS-722, RFuMS-823,RFuMS-824	-	+	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
53	**Gr1(Fu)-4:** NFuES-889,NFuES-890,BFuES-795,BFuES-796.	-	-	+
54	**Gr3(Fu)-18:** LFuES-729,LFuES-730,LFuES-731,LFuES-732, CFuES-761,CFuES-762,CFuES-763,CFuES-764,NFuES-891, NFuES-892,SFuES-859,SFuES-860,BFuES-793, BFuES-794, RFuES-825,RFuES-826,RFuES-827,RFuES-826	-	+	+
55	**Gr5(Fu)-14:** LFuSS-735,LFuSS-736,CFuSS-765,CFuSS-766, CFuSS-767,CFuSS-768,NFuSS-893,NFuSS-894,SFuSS-861, SFuSS-862, BFuSS-799, BFuSS-800,RFuES-831, RFuES-832	-	+	+
56	**Gr6(Fu)-20:** LFuMnS-1093,LFuMnS-1094,LFuMnS-1095, LFuMnS-1096,CFuMnS-1097, CFuMnS-1098,CFuMnS-1099, CFuMnS-1100,NFuMnS-1101,NFuMnS-1102, NFuMnS-1103, NFuMnS-1104,SFuMnS-1105,SFuMnS-1106,SFuMnS-1109, SFuMnS-1110,RFuMn-1113,RFuMn-1114	+	-	+
57	**Gr1(Ch)-48:** LChMS-741,LChMS-742,CChNS-769,CChNS-770, CChNS-771,CChNS-772, CChMS-775CChMS-776,NChNS-897, NChNS-898,NChNS-899,NChNS-900, NChMS-903,NChMS-904,SChNS-867,SChNS-868,BChMS-805,BChMS-806, BChMS-807,BChMS-808,RChNS-835,RChNS-836.LChMS-743,LChMS-744, CChMS-773,CChMS-774,NChMS-901, NChMS-902,SChMS-869,SChMS-870, SChMS-871,SChMS-872,BChNS-801,BChNS-802,BChNS-803,BChNS-804, RChMS-837,RChMS-838,RChMS-839,RChMS-840,LChNS-737, LChNS-738, LChNS-739,LChNS-740,SChNS-865,SChNS-866, RChNS-833,RChNS-834	-	+	+
58	**Gr8(Ch)-8:** LChES-747,LChES-748,NChES-907,NChES-908, SChES-873,SChES-874,BChES-811,BChES-818	-	-	+
59	**Gr2(Ch)-14:** CChES-779,CChES-780,CChSS-783,CChSS-784, BChSS-815, BChSS-816, LChSS-751,LChSS-752,CChES-781, CChES-782, SChSS-877,SChSS-878, RChSS-847, RChSS-848.	-	-	+

Table 4.16 Continue

Sl.	Group code and number of isolates	Gram staining	Motility	Catalase
60	**Gr2(Ch)-14:** CChSS-783,CChSS-784, NChSS-909,NChSS-910,SChSS-879,SChSS-880, RChES-841,RChES-842, LChSS-749,LChSS-750, NChSS-911,NChSS-912, BChSS-813,BChSS-814,	-	+	+
61	**Gr3(Ch)-12:** LChES-745,LChES-746,CChES-777,CChES-778, NChES-905,NChES-906,SChES-875,SChES-876,BChES-809, BChES-810,RChES-843, RChES-844	-	+	+
62	**Gr6(Ch)-16:** LChMnS-1117,LChMnS-1118,LChMnS-1119, LChMnS-1120,CChMnS-1121, CChMnS-1122,NChMnS-1125, NChMnS-1126,SChSS-1131,SChSS-1132,BChMn-1133, BChMn-1137,RChMn-1138, BChMn-1139,RChMn-1140	+	-	+

In the present study total 1140 isolates were isolated from different RTE food items for characterization and identification. Primarily all the isolates were tested for gram staining, motility and catalase. On the basis of gram staining, motality and catalase test results the isolates were grouped into 62 (Table 4.12). Then one representative isolate of each group was considered for further biochemical tests.

4.3.6 Biochemical test results of the selected isolates

Although morphological and cultural characteristics are essential for specific genus or species identification but it also requires more information. The physiological or biochemical experiment will be helpful for the identification of bacterial strains. For identification the following biochemical test performed according to Bergy's Manual and finally compare to ABIS and Microrao online software for confirmation of identification.

Catalase test results

Among the isolates, RKRM-211, CKRE-301, SMRM-651, SMRS-671, LCRE-22, LERE-101, CMRE-261, LCVN-5, LCVE-25, LTVN-45, RSVE-185, CKVM-295, CBVN-325, LCVS-35, SMVN-645, BAVM-416, BHVM-455, LCDM-19, BRDN-379, BADE-429, RKDE-229, LTDN-49, SMDM-659, LEDS-120, LCCM-13, LCCS-33, LECE-103, RCCM-133, RCCS-153, CMCN-243, SACM-693, SACS-713, BAFS-438, RKFE-227, CKFE-297, BAFM-417, RKFM-218, CMFM-257, CBFE-348, LCFS-37, CChNS-772, LChES-747, LChMS-744, NChES-906, SChSS-880, LCRMn-914, NTVMn-1015, SBCMn-1036, SAFMn-1067, LCDMn-919, CFuMn-1098, LChMn-1121 isolates showed positive catalase test results because it liberated gas (O_2) from hydrogen peroxide (H_2O_2) by enzymatic degradation (Table 4.12 - 4.18 and Plate 4.5D). Rest of the isolates showed negative results for catalase test.

Indole test results

Indole test demonstrates the ability of certain bacteria to split the amino acid tryptophan to indole which accumulates in the medium. Out of 62 isolates i.e. thirty seven isolates i.e. LERN-81, RKRM-211, CKRE-301, SMRM-651, LCRE-22, LERE-101, CMRE-261, LCVN-5, LCVE-25, LTVN-45, RSVE-185, CKVM-295, CBVN-325, BAVM-416, BHVM-455, LCDM-19, BADE-429, RKDE-229, LTDN-49, LTDS-79, BADN-409, SMDM-659, LCCM-13, LECE-103, RCCM-133, RCCS-153, SACM-693, SACS-713, RKFE-227, CKFE-297, RKFM-218, CMFM-257, CBFE-348, LChES-747, LChMS-744, NChES-906, SChSS-880 showed positive results for indole test and produced red color and rest of the isolates showed (yellow color) negative results (Table 4.13 - 4.20 and Plate 4.4 B).

Oxidase test results

The oxidase test (also known as the cytochrome oxidase test) was used to detect oxidase enzymes produced by certain bacteria. Oxidases catalyze electron transport between substrates acting as electron donors in the bacterium and tetra methyl-p-phenylenediamine or dimethyl-p-phenylenediamine- a redox dyes present as the hydrochloride or oxalate salt (the latter has a longer shelf-life). In this study LERN-81, RKRM-211, CKRE-301, LTVN-45, CBVN-325, SMVN-645, BAVM-416, BHVM-455, LCDM-19, BRDN-379,.LTDN-49, BADN-409, RCCM-133, RCCS-153, CMCN-243, BAFS-438, BAFM-417, CMFM-257, CChNS-772, LCChMS-744 isolates showed positive result and rest of the isolates showed negative result (Table 4.12 - 4.18 and Plate 4.5C).

Citrate utilization test results

Citrate utilization test was based on the ability of bacteria to use citrate as only carbon source and ammonia as its only source of nitrogen. Thirty one isolates i.e. LERN-81, RKRM-211, CKRE-301, LCRE-22, LCVN-5, LTVN-45,

CBVN-325, SMVN-645, BAVM-416, BHVM-455, LCDM-19, BADE-429, LTDN-49, LTDS-79, BADN-409, LCCM-13, RCCM-133, RCCS-153, SACM-693, RKFE-227, CKFE-297, CMFM-257, LChMS-744, SChSS-880, LCRMn-914, NTVMn-1015, SBCMn-1036, SAFMn-1067, LCDMn-919, CFuMn-1098, LChMn-1121 showed positive result and produce blue color (Table 4.12 - 4.18 and plate - 4.5A) but rest of the isolates showed negative results and produce yellow color.

Methyl red test and Voges-Proskauer test results

Methyl red test

This test was performed to identify mixed acid fomenter bacteria. In this case thirty one isolates i.e. CKRE-301, SMRM-651, LERE-101, CMRE-261, LCVN-5, LTVN-45, RSVE-185, CKVN-295, SMVN-645, BHVM-455, LCDE-19, RKDE-229, LTDN-49, LTDS-79, BADN-409, SMDN-659, LEDS-120, LCCM-13, LCCS-33, LECE-103, RCCM-133, RCCS-153, SACM-693, RKFM-227, RKFM-218, CMFM-257, CBFE-348, LCFS-37, LChMS-744, NChES-906, SChSS-880 showed positive result by the appearance of a bright red color, indicating acidity and rest of the isolates showed negative methyl red test by producing yellow or orange color indicating alkalinity (Table 4.12- 4.18 and plate-4.5B).

Voges-Proskauer test

This test was done to detect the production of acetylene, due to the development of a red ring at the surface of the medium. In this case twenty two isolates i.e. LERE-81, LCRE-22, LCVE-25, SMVN-645, BRDN-379, BADE-429, BADN-409, CMCN-243, SACS-713, BAFS-438, CKFE-297, BAFM-417, CKFE-297 , CChNS-772, LChES-747, LCRMn-914, NTVMn-1015, SBCMn-1036, SAFMn-1067, LCDMn-919, CFuMnS-1098, LChMn-1121 showed positive VP test results (Table 4.12- 4.18 and plate-4.5C)

and rest of the isolates showed negative VP test because these bacteria produced acetylene.

Phenylalanine test results

In this case LERN-81, RKRM-211, SMRM-651 ,CBVN-325, SMVN-645 ,BAVM-416, BADN-409, SMDM-659, LCCM-13, CKFE-297 isolates showed positive result but rest of the isolates showed negative result (Table 4.12- 4.18 and plate 4.5D).

Triple Sugar Iron (TSI) test results

Triple Sugar Iron (TSI) was used in the identification of Gram negative bacteria particularly the Enterobacteriaceae. TSI reactions were based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide. All the isolates showed yellow color in TSI butt. Twenty eight isolates i.e. LChMS-744, CFuSS-754, BFuSS-796, BAFS-438 , RKFE-227, BAFE-417, RKFM-218, RCCM-133, RCCS-153, CMCN-243, SACM-693, SACS-713, LTDN-49, LCVE-25, LTVN-45, CBVN-325, SMVN-645, BAVM-416, BHVM-455, RKRM-211, CKRE-301, LCRMn-914, NTVMn-1015 SBCMn-1036, SAFMn-1067,LCDMn-919, CFuMnS-1098, LChMn-1121 isolates showed red color in TSI slant and rest of the isolates showed yellow color in TSI butt. Eight isolates i.e.LChS-747, NChES-906, LChES-747, LECE-103, BRRN-379, BADE-429, RKDE-229, LCRE-22 showed positive result for H_2S and produced black color and rest of the isolates are H_2S negative (Table 4.12- 4.18 and plate 4.5E.)

Strach hydrolysis test results

Strach agar is a differential medium that test the abili[illegible] an organ[illegible] produce certain exo-enzyme, including a-amylas[illegible] oligo[illegible] glocosidase, that hydrolyze starch. Since no colour chan[illegible]s in th[illegible]

medium when organism hydrolyze starch, after added iodine to the plate after incubation. Iodine turns blue, purple or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolyze starch incase of starch hydrolysis test. LCRMn-914, NTVMn-1015, SBCMn-1036, SAFMn-1067, LCDMn-919, CFuMnS-1098, LChMnS-1121 isolates showed negative. (Table – 4.18 Plate 4.5F) and rest of the isolates showed positive result.

Coagulase test results

Coagulase test is used to differentiate *Staphyloccous aureus* (positive) from coagulase. Negative *Staphyloccous* (CONS) Coagulase is an enzyme produce by *Staphyloccus aureus* that covert (soluble) fibrinogen in plasma to (insoluble) fibrin. *Staphyloccus aureus* produces to from of coagulase bound and free. In this case LCRMn-914 ,NTVMn-1015, SBCMn-1036 , SAFMn-1067, LCDMn-919, CFuMnS-1098, LChMnS-1121 isolates showed positive (Table 4.18, Plate 4.5G) and rest of the isolates showed positive result.

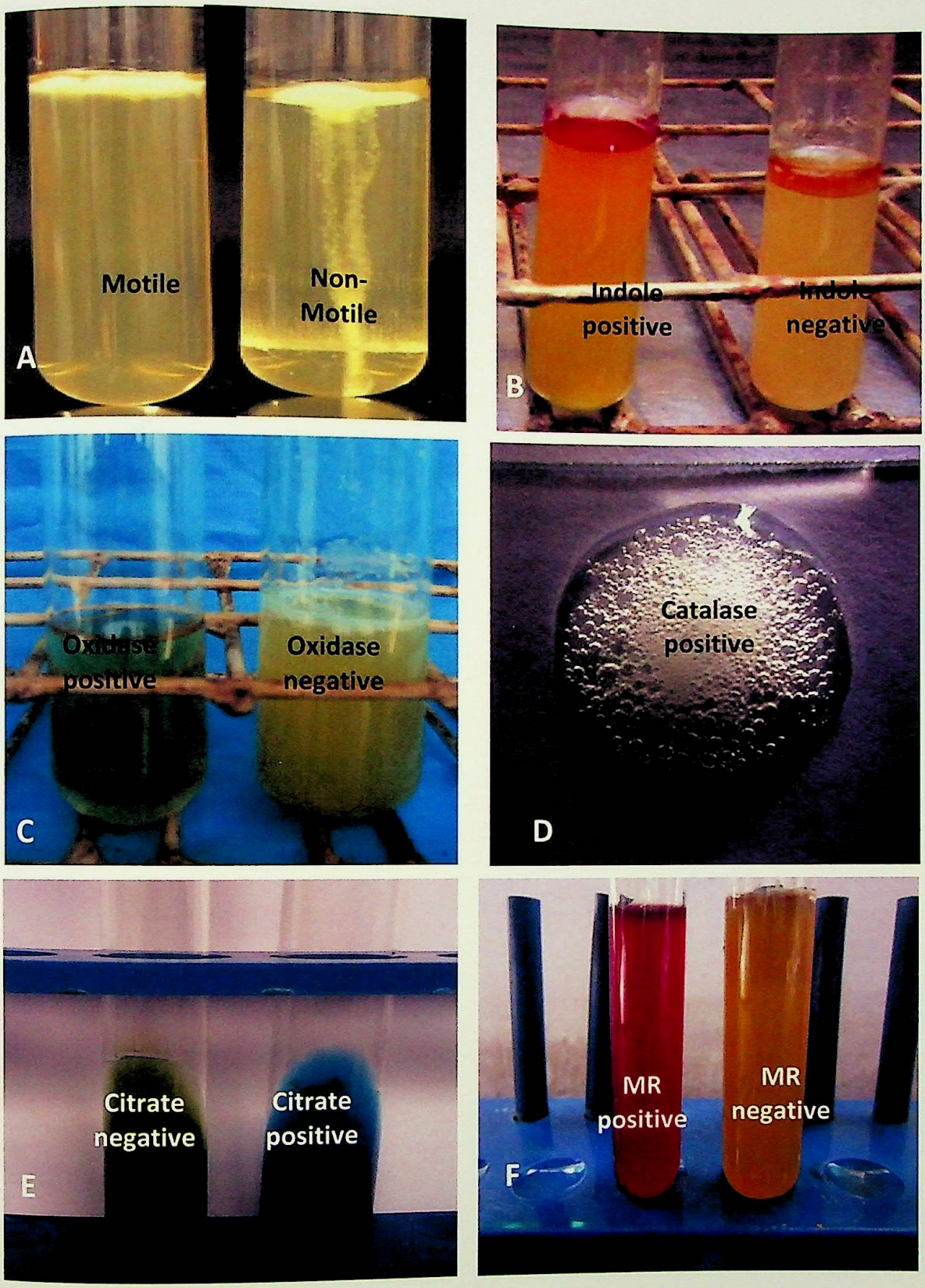

Plate 4.6. Photographs showing different biochemical test results (A-F) of the selected isolates.

Plate 4.7. Photographs showing different biochemical test results (A-F) of the selected isolates.

tequilensis (SMVN-645) with the conformity of 95%, *Proteus vulgaris* (BAVM-416) with the conformity of 98%; *Citrobacter kosery* (BHVM-455) with the conformity of 98%.

From the Table 4.19 it was observed that in cooked dal, total nine isolates were tested and they were finally identified as *Citrobacter kosewri* (LCDM-19) with the conformity of 98%, *Enterobacter aerogene* (BRRN-379) with the conformity of 99.9% , *Klebseilla oxytoca (* BADE-4 29) with the conformity of 98%, *Escherichia coli* (RKDE-229) with the conformity of 99%, *Citrobacter koseri* (LTDN-49) with the conformity of 88%; *Citrobacter frundii* (LTDS-79) with the conformity of 90.4%, *Bacillus tequilensis* (BADN-409) with the conformity of 95%, *Proteus vulgarir*s (SMDM-659) with the conformity of 98%, *Salmonella typhi* (LEDS-120) with the conformity of 96.8%.

From the Table 4.20 it was observed that in chicken curry, total eight isolates were tested and they were finally identified as *Proteus vulgarir*s (LCCM-13) with the conformity of 98%, *Salmonella typhi* (LCCS-33) with the conformity of 96.8%, *Esherichia coli* (LECE-103) with the conformity of 99%, *Citrobacter frundii* (RCCM-133) with the conformity of 92.01%, *Citrobacter koseri* (RCCS-153) with the conformity of 88%, *Enterobacter aerogene s(*CMCN-243) with the conformity of 89.9% , *Proveidencia alcalifaciens* (SACM-693) with the conformity of 99%, and *Klebseilla oxytoca* (SACS-713) with the conformity of 99.97%.

From the Table 4.21 it was observed that in fish curry, total eight isolates were tested and they were finally identified as *Enterobacter aerogenes* (BAFS-438) with the conformity of 89.9%, *Proveidencia alcalifaciens* (RKFE-227) with the conformity of 99%, *Proteus mirabikis* (CKFE-297) with the conformity of 91.1%, *Enterobacter aerogenes* (BAFM-417) with

the conformity of 79.9%, *Edwardseilla tarda* (RKFE-218) with the conformity of 99%, *Citrobacter frundii* (CMFM-257) with the conformity of 92.1%, *Esherichia coli* (CBFE-348) with the conformity of 99% and *Salmonella typhi* (LCFS-37) with the conformity of 96.8%.

From the Table 4.22 it was observed that in fuchka total five isolates were tested and they were finally identified as Enterobacter *aero genes* (CFuSS-754) with the conformity of 89.9%, *Klebseilla oxytoca (* BFuSS-796) with the conformity of 99.97%, *Esherichia coli* (RFuES-828) with the conformity of 99%, *Citrobacter kosery* (RFuSS-829) with the conformity of 88%, *Salmonella typhi* (CFuSS-766)with the conformity of 86.8%.

From the Table 4.23 it was observed that in chatpoti, total five isolates were tested and they were finally identified as Enterobacter *aerogenes* (CChNS-772) with the conformity of 89.9%, *Klebseilla oxytoca* (LChES-747) with the conformity of 98%, *Citrobacter kosery* (LChMS-744) with the conformity of 88%, *Esherichia coli* (NChES-906) with the conformity of 99%, *Citrobacter frundii* (SChSS-906) with the conformity of 97.4%, Only seven isolates i.e.LCRMn-914, LCDMn-910, NTVMn-1015, SAFMn-1067, SBCMn-1036, CFuMnS-1098, LChMnS-1121 isolated from MS medium were finally identified as *Staphylococcus aureus* with the conformity of 89.6% (Table 4.24).

Finally, the identified bacteria in different RTE food items are summarized (Table 14.25) which were identified as ten genera as below:

- *Proteus*
- *Edwardsiella*
- *Klebsiella*
- *Salmonella*
- *Escherichia*
- *Citrobacter*
- *Enterbacter*
- *Bacillus*
- *Staphyloccous*
- *Providencia*

Table 4.18. Results of standard biochemical tests for identification of the selected bacterial isolates of vegetable curry

Group code & total number of isolates	Biochemical test results											Suspected bacteria (% of conformity)
	Motility	Catalase	Indole	Methyl red	Vogues Proskauer	Phenylalanine	Oxidase	Citrate	TSI			
									Stant	Butt	H_2S	
LCVN-5	-	+	+	+	-	-	-	+	R	Y	+	*Citrobacter frundii-* (92.1%)
LCVE-25	-	+	+	-	+	-	-	-	R	Y	-	*Klebseilla oxytoca* (99.97%
LTVN-45	+	+	+	+	-	-	+	+	R	Y	-	*Citrobacter kosery –* (98%)
RSVE-185	+	+	+	+	-	-	-	-	Y	Y	+	*Esherichia coli-*(99%)
CBVN-295	+	+	+	+	-	-	-	-	R	Y	+	*Edwardsiella tarda-* (99%)
CBVN-325	+	+	+	-	-	+	+	+	R	Y	-	*Enterobacter aerogenes-(99.9%)*
LCVS-35	+	+	-	-	-	-	-	-	R	Y	+	*Salmonella typhi-* (96.8%)
SMVN-645	+	+	-	+	+	+	+	+	R	Y	-	*Bcillus tequilensis* (75%)
BAVM-416	+	+	+	-	-	+	+	+	R	Y	-	*Proteus vulgaris(98%)*
BHVM-455	+	+	+	+	-	-	+	+	R	Y	-	*Citrobacter kosery-* (98%)

[Key: Gr= Group, TSI-Triple sugar iron, H_2S =Hydrogen sulphyde, R=Red, Y=Yellow, '+'=Positive, '-'= Negative. Conformation was conducted by using ABIS online bacterial identification software .]

Table 4.19. Results of standard biochemical tests for identification of the selected bacterial isolates of cooked dal

Group code & total number of isolates	Biochemical test results											Suspected bacteria (% of conformity)
	Motility	Catalase	Indole	Methyl red	Vogues Proskauer	Phenylalanin	Oxidase	Citrate	TSI			
									Stant	Butt	H_2S	
LCDM-19	-	+	+	+	-	-	+	+	R	Y	+	*Citrobacter koseri-(98%)*
BRRN-379	+	+	-	-	+	-	+	-	Y	Y	-	*Enterobacter* aerogenes-(99.9%)
BADE-429	-	+	+	-	+	-	-	+	Y	y	-	*Klebseilla oxytoca-(98%)*
RKDE-229	+	+	+	+	-	-	-	-	Y	Y	-	*Escherichia Coli-(99%)*
LTDN-49	+	+	+	+	-	-	+	+	R	Y	-	*Citrobacer koseri-(98%)*
LTDS-79	-	+	+	+	-	-	-	+	R	Y	+	*Citrobacer frundii-(90%)*
BADN-409	-	-	+	+	+	+	+	+	R	Y	+	*Bacillus tequilensis-(95%)*
SMDM-659	+	+	+	+	-	+	-	-	Y	Y	+	*Proteus vulgaris-(98%)*
LEDS-120	+	+	-	+	-	-	-	-	R	Y	+	*Salmonella typhi* – (96.8%)
BRDT-1063	+	+	+	+	-	-	-	-	R	Y	+	*Edwardseilla tarda-(99%)*

[Key: Gr= Group, TSI-Triple sugar iron, H_2S =Hydrogen sulphyde, R=Red, Y=Yellow, '+'=Positive, '-'= Negative. Conformation was conducted by using ABIS online bacterial identification software.]

Table 4.20. Results of standard biochemical tests for identification of the selected bacterial isolates of chicken curry

Group code & total number isolates	Biochemical test results											Suspected bacteria (% of conformity)
	Motility	Catalase	Indole	Methyl red	Vogues Proskauer	Phenylalanine	Oxidase	Citrate	TSI			
									Stant	Butt	H_2S	
LCCM-13	+	+	+	+	-	+	-	+	R	Y	+	*Proteus vulgaris-(98%)*
LCCS-33	+	+	-	+	-	-	-	-	R	Y	+	*Salmonella typhi-(98.8%)*
LECE-103	+	+	+	+	-	-	-	-	Y	Y	-	*Escherichia coli-*(99%)
RCCM-133	+	+	+	+	-	-	+	+	R	Y	-	*Citrobacter frundii-*(92.01%)
RCCS-153	+	+	+	+	-	-	+	+	R	Y	-	*Citrobacter koseri-(88%)*
CMCN-243	+	+	-	-	+	-	+	-	R	Y	-	*Enterobacter aerogenes-(89.9%)*
SACM-693	+	+	+	+	-	-	-	+	R	Y	-	*Proveidencia alcalifaciens-*(99%)
SACS-713	-	+	+	-	+	-	-	-	R	Y	-	*Klebseilla oxytoca-*(99.97%)
NTCM-493	+	+	+	+	-	-	-	-	R	Y	+	*Edwardseilla tarda-(99%)*

[Key: Gr= Group, TSI-Triple sugar iron, H_2S =Hydrogen sulphyde, R=Red, Y=Yellow, '+'=Positive, '-'= Negative. Conformation was conducted by using ABIS bacterial identification software.]

Table 4.21. Results of standard biochemical tests for identification of the selected bacterial isolates of fish curry

Group code & total number isolates	Biochemical test results											Suspected bacteria (% of conformity)
	Motility	Catalase	Indole	Methyl red	Vogues Proskauer	Phenylalanine	Oxidase	Citrate	TSI			
									Stant	Butt	H_2S	
BAFS-438	+	+	-	-	+	-	+	-	R	Y	-	*Enterobacter aerogenes-(89.9%)*
RKFE-227	+	+	+	+	-	-	-	+	R	Y	-	*Proveidincia alcalifaciens.(-99%)*
CKFE-297	+	+	+	-	+	+	-	+	R	Y	+	*Proteus mirabilis (91.1%)*
BAFM-417	+	+	-	-	+	-	+	-	R	Y	-	*Enterobacter aerogenes*-(79.9%)
RKFE-218	+	+	+	+	-	-	-	-	R	Y	-	*Edwarseilla tarda*-(99%)
CMFM-257	-	+	+	+	-	-	+	+	R	Y	+	*Citrobacter frundii-(92.1%*)
CBFE-348	+	+	+	+	-	-	-	-	Y	Y	+	*Escherichia coli-(99%)*
LCFS-37	+	+	-	+	-	-	-	-	R	Y	+	*Salmonella typhi*-(96.8%)

[Key: Gr= Group, TSI-Triple sugar iron, H_2S =Hydrogen sulphyde, R=Red, Y=Yellow, '+'=Positive, '-'= Negative. Conformation was conducted by using ABIS bacterial identification software.]

Table 4.22: Results of standard biochemical tests for identification of the selected bacterial isolates of fuchka

Group code & total number of isolates	Biochemical test results											Suspected bacteria (% of conformity)
	Motility	Catalase	Indole	Methyl red	Vogues Proskauer	Phenylalanine	Oxidase	Citrate	TSI			
									Stant	Butt	H_2S	
CFuSS-754	+	+	-	-	+	-	+	-	R	Y	-	*Enterobacter aerogenes(-89.9%)*
BFuSS-796	-	+	+	-	+	-	-	-	R	Y	-	*Klebseilla oxytoca-(99.97%)*
RFuES-828	+	+	+	+	-	-	-	-	Y	Y	+	*Escherichia coli-(99%)*
RFuSS-829	-	+	+	+	-	-	+	+	R	Y	+	*Citobacter kosery-(88%)*
CFuSS-766	+	+	-	+	-	-	-	-	R	Y	+	*Salmonella typhi.-(86.8%)*

[Key: Gr= Group, TSI-Triple sugar iron, H_2S =Hydrogen sulphyde, R=Red, Y=Yellow, '+'=Positive, '-'= Negative. Conformation was conducted by using ABIS bacteria identification software]

Table 4.23. Results of standard biochemical tests for identification of the selected bacterial isolates of chatpoti

Group code & total number isolates	Biochemical test results											Suspected bacteria (% of conformity)
	Motility	Catalase	Indole	Methyl red	Vogues Proskauer	Phenylalanine	Oxidase	Citrate	TSI			
									Stant	Butt	H_2S	
CChNS-772	+	+	-	-	+	-	+	-	R	Y	+-	*Enterobacter aerogenes-(79.9%)*
LChES-747	-	+	+	-	+	-	-	-	Y	Y	-	*klebsiella oxytoca-(98%)*
LChMS-744	+	+	+	+	-	-	+	+	R	Y	-	*Citrobacter kosery-(98%.)*
NChES-906	+	+	+	+	-	-	-	-	Y	Y	-	*Escherichia coli-(99%)*
SChSS-880	-	+	+	+	-	-	-	+	R	Y	+	*Citrobacter frundii-(97.4%)*

[Key: Gr= Group, TSI-Triple sugar iron, H_2S =Hydrogen sulphyde, R=Red, Y=Yellow, '+'=Positive, '-'= Negative. Conformation was conducted by using ABIS bacteria identification software.]

Table 4.24. Results of standard biochemical tests for identification of selected bacterial isolates

Group code & total number isolates	Catatalase	Inodole	Vogues Proskauer	Phenylalanine	Oxidase	Citrate	Coagulage	Starch	TSI			Suspected bacteria (% of conformity)
									Stant	Butt	H_2S	
LCRMn-914, LCDMn-910, NTVMn-1015, SAFMn-1067, SBCMn-1036, CFuMnS-1098, LChMnS-1121	+	-	+	-	-	+	+	-	R	Y	-	*Staphylococcus aureus-(*89.6%)

[Key: Gr= Group, TSI-Triple sugar iron, H_2S =Hydrogen sulphyde, R=Red, Y=Yellow, '+'=Positive, '-'= Negative. Conformation was conducted by using Micro Rao bacteria identification software.]

Table 4.25. Types of presumptive bacteria in boiled rice, vegetable curry, chicken curry, fish curry, fuchka and chatpoti

Boiled rice	Vegetable curry	Chicken curry	Fish curry	Cooked Dal	Fuchka	Chatpoti
Bacillus tequilens	*Citrobacter frundii*	*Enterobacter aerogenes*	*Proteus mirabilis*	*Proteus vulgaris*	*Enterobacter aerogenes*	*Enterobacter aerogenes*
Citrobacter koseri	*Klebsiella oxytoca*	*Salmonella typhi*	*Proveidencia Alcalifaciens*	*Klebsiella oxytoca*	*Citrobacter koseri*	*Klebsiella oxytoca*
Proteus vulgaris	*Escherichia coli*	*Escherichia coli*	*Enterobacter aerogenes*	*Citrobacter frundii*	*Klebsiella oxytoca*	*Citrobacter koseri*
Salmonella typhi	*Edwardsiella tarda*	*Citrobacter frundii*	*Edwardsiella tarda*	*Bacillus tequilensis*	*Escherichia coli*	*Citrobacter frundii*
Edwardsiella tarda	*Proteus vulgaris*	*Citrobacter koseri*	*Citrobacter frundii*	*Enterobacter aerogenes*	*Salmonella typhi*	*E. coli*
Klebseilla oxytoca	*Salmonella typhi*	*Proteus vulgaris*	*Escherichia coli*	*Citrobacter koseri*	*Staphyloccous* sp	*Staphyloccous* sp
Enterobacter	*Bacillus tequilensis*	*Proveidencia alcalifaciens*	*Klebsiella oxytoca*	*Escherichia coli*		
aerogenes	*Enterobacter aerogenes*	*Klebsiella oxytoca*	*Salmonella typhi*	*Salmonella typhi*		
Escherichia coli	*Citrobacter koseri*	*Staphyloccous* sp	*Staphyloccous* sp	*Staphyloccous* sp		
Staphyloccous sp	*Staphyloccous* sp					

4.4. Detection of bacterial diversity among the RTE food items sold in Rjshahi Metroplolis

4.4.1 Frequency of different bacteria in different RTE food items

Total 570 RTE food samples namely boiled rice, cocked dal, vegetables curry, fish curry, chicken curry, fuchka and chatpoti of Rajshahi Metropolis were tested. These isolates were identified as ten genera i.e *Proteus* sp, *Edwarsiella* sp., *Escherichia coli, Citrobacter* sp., *Klebseilla* sp., *Enterobacter* sp., *Salmonella* sp., *Provedencia* sp., *Bacillus* sp. and *Staphylococcus* sp. The frequency of these genera with the samples were determined and the results are presented in Table 4.26.

For boiled rice, the maximum frequency was recorded for *Enterobacter* sp. (42.2%) and the lowest was *Edwarsiella* sp.(8.8%). Moderated frequency were recorded as 33.33%, 17.7%, 13.5%, 13.3% and 2.22% for *Citrobacter* sp., *Bacillus* sp., *Proteus* sp., *Klebseilla* sp., *Salmonella* sp., *E.coli, Staphylococcus* sp, *Bacillus* sp, respectively. *Provedencia* sp. was not detected.

For cooked dal, the highest frequency (53.3%) was recorded for *Citrobacter* sp. and the lowest was *Bacillus* sp (3.3%). Modareted frequency were recorded as 38.8%, 35.5%, 17.7%, 15.5%, 15.5%, 10% and 6.6%, respectively for *Enterobacter* sp., *Salmonella* sp., *E.coli, Edwarsiella* sp., *Klebseilla* sp., *proteus* sp. and *Staphylococcus* sp. respectively. *Provedencia* sp. was not detected.

For vegetable curry, the highest frequency (64.4%) was detected for of *Citrobacter* sp. and the lowest was *Edwarsiella* sp. (4.4%). The moderate frequency were recorded as 50.3%, 17.7%, 15.5%, 14.4%, 13.3%, 13.3% and 12.2%, respectively for *Enterobacter* sp., *Klebseilla* sp., *Staphylococcus* sp., *E.coli, Salmonella* sp., *Bacillus* sp. and *Proteus* sp., *respectively. Provedencia* sp. was absent in vegetables curry.

For fish curry, the highest frequency (64.4%) was recorded for *Proteus* sp. and the lowest was *Staphylococcus* sp (11.11%). Moderate frequency were recorded as 46.6%, 24.4%, 24.4%, 20%, 19.56% and 13.3% for *Enterobacter* sp., *E.coli, Citrobacter* sp., *Salmonella* sp., *Provedencia* sp. and *Edwarseilla* sp, respectively. *Bacillus* sp. and *Klebseilla* sp. were not detected.

For chicken curry, the highest frequency was recorded for *Citrobacter* sp. (53.3%) and the lowest was *Edwarseilla* sp.(4.4%). The modared frequency were recorded as 22.22%, 21.1%, 20%, 17.7%, 15.5%, 13.3% and 13.3% for *Salmonella* sp., *Provedencia* sp., *E.coli, Proteus* sp., *Staphylococcus* sp., *Klebseilla* sp. and *Enterobacter* sp., respectively *Bacillus* sp not detected.

For fuchka , the highest frequency was recorded for *Staphylococcus* sp. (70%) and the lowest was *Klebseilla* sp.(6.6%) . The moderate frequency were recorded as 50%, 30%, 30% and 23.3% for *Enterobacter* sp., *E.coli, Citrobacter* sp. and *Salmonella* sp., respectively. *Proteus* sp., *Edwarseilla* sp., *Bacillus* sp. and *Provedencia* sp. were not detected.

For chatpoti, the highest frequency was detected for of *Enterobacter* sp. (80%) and the lowest was *Staphylococus* sp. (2.33). The moderate frequency were recorded as 70%, 20% and 13.3% for *Citrobacter* sp., *E. coli.* and *Klebseilla* sp., respectively. *Proteus* sp., *Edwarseilla* sp., *Salmonella* sp., *Bacillus* sp. and *Provedencia* sp. were not detected.

In overall, it was observed that the most prevalent genera was *Ctrorobacter*(46.6%). Next of *Enterobacter* (44.38%), *Staphylococcus* (19.64%), *E.coli*(19.47%), *Salmonella* (18.94%),*Proteus* (17.54%), *Klebseilla* (11.57%) *Edwaeseilla* (7.36%), *Provedencia* (6.49%). And the lowest frequency was *Bacillus* (6.14).

Table 4.26 Frequency of bacteria in different RTE foods

Name of bacteria	Boiled Rice n=90	Cocked Dal n=90	Vegetables curry n=90	Fish curry n=90	Chicken curry n=90	Fuchka n=60	Chatpoti n=60	Total isolates	Total frequrncy (%) N=570
Proteus sp.	*16(17.7%)	9 (10%)	11 (12.2%)	58(64.4%)	16(17.7%)	-	-	100	17.54
Edwarseilla sp.	8(8.8%)	14 (15.5)	4 (4.4%)	12(13.3%)	4 (4.4%)	-	-	42	7.36
Escherichia coli	12(13.5%)	16(17.7%)	13(14.4%)	22 (24.4%)	18 (20.0%)	18 (30%)	12 (20%)	111	19.47
Citrobacter sp.	30 (33.3%)	48 (53.3%)	58 (64.4%)	22 (24.4%)	48 (53.3%)	18 (30%)	42 (70%)	266	46.6
Klebseilla sp	12 (13.3%)	14 (15.5%)	16(17.7%)	-	12(13.3%)	4 (6.6%)	8 (13.3%)	66	11.57
Enterobacter sp.	38 (42.2%)	35 (38.8%)	48 (50.3%)	42 (46.6%)	12(13.3%)	30 (50%)	48 (80%)	253	44.38
Salmonella sp.	12 (13.3%)	32(35.5%)	12(13.3%)	18 (20%)	20(22.2%)	14 (23.3%)	-	108	18.94
Provedencia sp.	-	-	-	18 (19.56%)	19 (21.1%)	-	-	37	6.49
Bacillus sp	20(2.22%)	3(3.3%)	12(13.3%)	-	-	-	-	35	6.14
Staphylococcus sp.	12 (13.3%)	6(6.6%)	14(15.5%)	10(11.11%)	14 (15.5%)	42(70%)	14(2.33 %)	112	19.64

n= Number of samples, N= Total samples, * =number of isolates

4.4.2 Abundance of different bacteria in different RTE food items

In the present study total 1140 bacterial isolates were isolated from the different RTE food items sold in Rajshahi Metropolis. The isolates were identified as ten genera i.e. *Proteus* sp., *Edwarseilla* sp., *E. coli*, *Citrobacter* sp., *Klebseilla* sp., *Enterobacter* sp, *Salmonella* sp., *Provedencia* sp, *Bacillus* sp. and *Staphyloccus* sp. The abundance of these genera in different food items were determined and the results are presented in Fig 4.9-4.16.

Boiled rice samples contained total nine genera of the bacter*ia namely* *Proteus* sp., *Edwarseilla* sp., *E. coli*, *Citrobacter* sp. *Klebseilla* sp., *Enterobacter* sp, *Salmonella* sp., *Bacillus* sp. and *Staphyloccus* sp.The prevalence of the bacteria were 23.75% for *Enterobacter*, 18.75 for *Citrobacter* sp., 12.5% for *Bacillus* sp., 10% for *Proteus* sp.; 7.5% for *E. coli, Klebseilla* sp, *Salmonella* sp., *and* *Staphyloccus*; and 5% for *Edwarseilla* sp. The most three dominant bacteria were *Enterobacter*, *Citrobacter* and *Bacillus* and which contributed 55% of the total bacteria in boiled rice.

Cooked dal samples contained total nine genera of the bacter*ia namely* *Proteus* sp., *Edwarseilla* sp., *E. coli, Citrobacter* sp. *Klebseilla* sp., *Enterobacter* sp, *Salmonella* sp., *Bacillus* sp. and *Staphyloccus* sp. The prevalence of the bacteria were 27.11% for *Citrobacter*, 19.77 for *Enterobacter* sp., 18.1% for *Salmonella* sp., 9.03% for *E.coli*, 9.03 *Klebseilla* sp, *Edwarseilla* sp, 5.01% for *Proteus* sp, 3.38 % for *Staphylococcus* and 1.9% for *Bacillus* sp. The most three dominant bacteria were *Enterobacter, Citrobacter* and *Salmonella* which contributed 64.98% of the total bacteria in cooked dal.

Vegetable curry samples contained total nine genera of the bacter*ia* namely *Proteus* sp., *Edwarseilla* sp., *E. coli, Citrobacter* sp, *Klebseilla* sp.,

Enterobacter sp, *Salmonella* sp., *Bacillus* sp. and *Staphyloccus* sp. The prevalence of the bacteria were 30.85% for *Citrobacter*, 25.53% for *Enterobacter* sp., 8.51% for *Klebseilla* sp, 7.44% for *Staphylococcus* sp, 6.91% for *E. coli*; 6.38% for *Salmonella* sp. and *Bacillus* sp.; 5.85% for *Proteus* sp, and 2.1% for *Edwarseilla* sp. The most three dominant bacteria were *Enterobacter* sp, *Citrobacter* sp and *E. coli* which contributed 85.75% of the total bacteria in vegetable curry.

Fish curry samples contained total eight genera of the bacter*ia namely Proteus* sp., *Edwarseilla* sp., *E. coli, Citrobacter* sp. *Enterobacter* sp, *Salmonella* sp., *Bacillus* sp. and *Staphyloccus* sp. The prevalence of the bacteria were 28.71% for *Proteus* sp, 20.79% for *Enterobacte*r sp., 10.79% for *E. coli* and *Citrobacter* 8.91% for *Salmonella* sp, 8.9% for *Proveidencia* sp, 5.94% for *Edwarseilla* sp, 4.95% for *Staphylococcus* sp. The most three dominant bacteria were *Proteus* sp, *Enterobacter* sp, and *Citrobacter* sp which contributed 60.38% of the total bacteria in fish curry .

Chicken curry samples contained total nine genera of the bacter*ia namely Proteus* sp., *Edwarseilla* sp., *E. coli, Citrobacter* sp., *Enterobacter* sp, *Salmonella* sp., *Klebseilla* sp, *Provedencia* sp and *Stapphyloccus* sp. The prevalence of the bacteria were 29.44% for *Citrobacter* sp, 12.26% for *Salmonella* sp., 11.65% for *Provedencia* sp, 11.1% for *E. coli,* 9.81% for *Proteus* sp., 8.58% for *Stpphylococcus* sp; 7.36% for *Klebseilla and Enterobacter* sp; and 2.45% for *Edwarseilla* sp. The most three dominant bacteria were *Citrobacter* sp, *Salmonella* sp and *Provedencia* sp which contributed 53.35% of the total bacteria in chicken curry .

Fuchka samples contained total six genera of the bacter*ia namely E. coli, Citrobacter* sp, *Enterobacter* sp, *Salmonella* sp., *Klebseilla* sp and *Stapphyloccus* sp. The prevalence of the bacteria were 33.3% *for Staphylococcus* sp, 23.08% for *Enterobacte*r sp; 14.28% *E. coli* and

Citrobacter sp; and 11.1 % for *Salmonella* sp. and 3.17% for *Klebseilla* sp. The most three dominant bacteria were *Enteobacter* sp , *Staphylococus* sp *and Citrobacter* sp which contributed 71.38% of the total bacteria in Ffuchka.

Chatpoti samples contained total five genera of the bacter*ia namely E. coli*, *Citrobacter* sp. *Enterobacter* sp, *Klebseilla* sp and *Staphyloccus* sp. The prevalence of the bacteria were 38.7% for *Enteobacter* sp, 33.87% for C*itroboracter* sp, 11.3% for Staphylococcus sp, 9.67% for *E. coli*, 6.45% f*Klebseilla* sp. The most three dominant bacteria were *Citrobacter* sp, *Enteobacter* sp and *Staphyloccus* which contributed 83.87% of the total nt

In overall bacterial distribution, it was observed that *Citrobacter* sp was the most frequent genera of RTE food samples sold in Rajshahi Metropolis and showed 23.3% of total abundance. Next of most dominating bacteria were *Enterobacter* (22.19%), *Salmonella* sp (9.47%), *Proteus* sp (8.7%), *Klebseilla* sp (5.78%), *Edwarseilla* sp (3.68%), for *Proveidencia* sp(3.24%). The least dominanting bacteria was *Bacillus* sp (3.07%). On the other hand, only four genera i.e. *Enterobacter* sp, *Citrobacter* sp, *E. coli* and *Staphylococcus* sp were the most prevalence bacteria found in all types of RTE food items. *Salmonella* sp was absent in chatpoti, *Proteus* sp was absent in fuchka and chatpoti; *Klebsilla* sp was absent in fish curry; *Proveidencia* sp was absent in boiled rice, cooked dal, vegetable curry, fuchka and chatpoti; and *Bacillus* sp absent in fish curry, chicken curry, fuchka and chatpoti.

The rank of the bacteria for total abundance were *Ctrobacter* > *Enterobacter* > *Staphylococcus*> *E.coli*> *Salmonella*> *Proteus*> *Klebseilla* >*Edwarsilla* > *Provedencia*> *Bacillus*

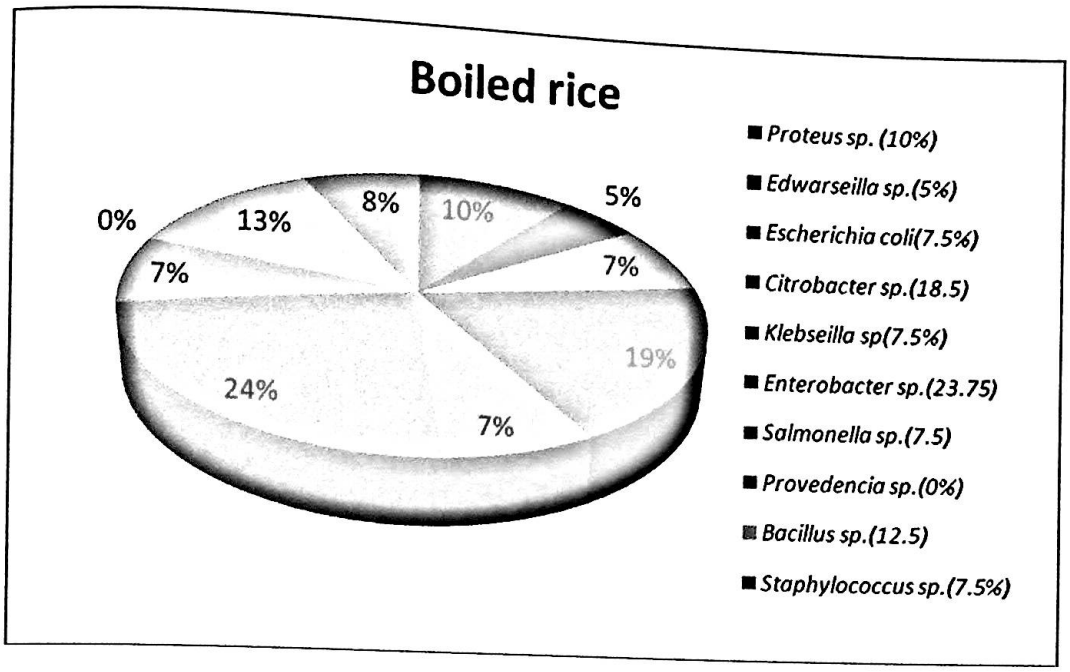

Fig 4.9. Abundance of different bacteria in boiled rice.

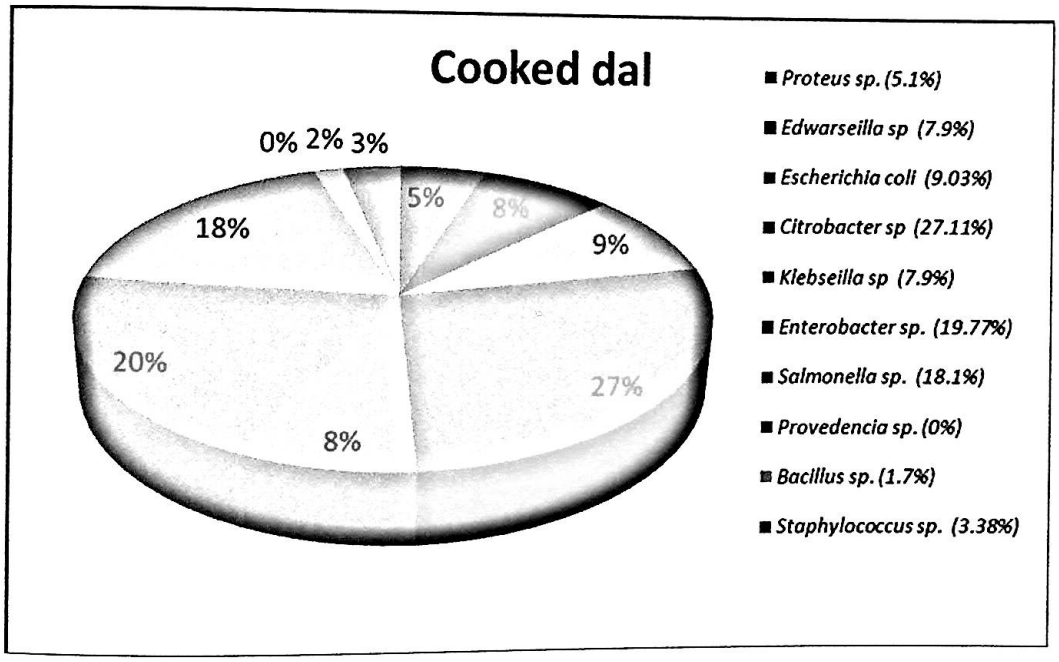

Fig 4.10. Abundance of different bacteria in cocked dal.

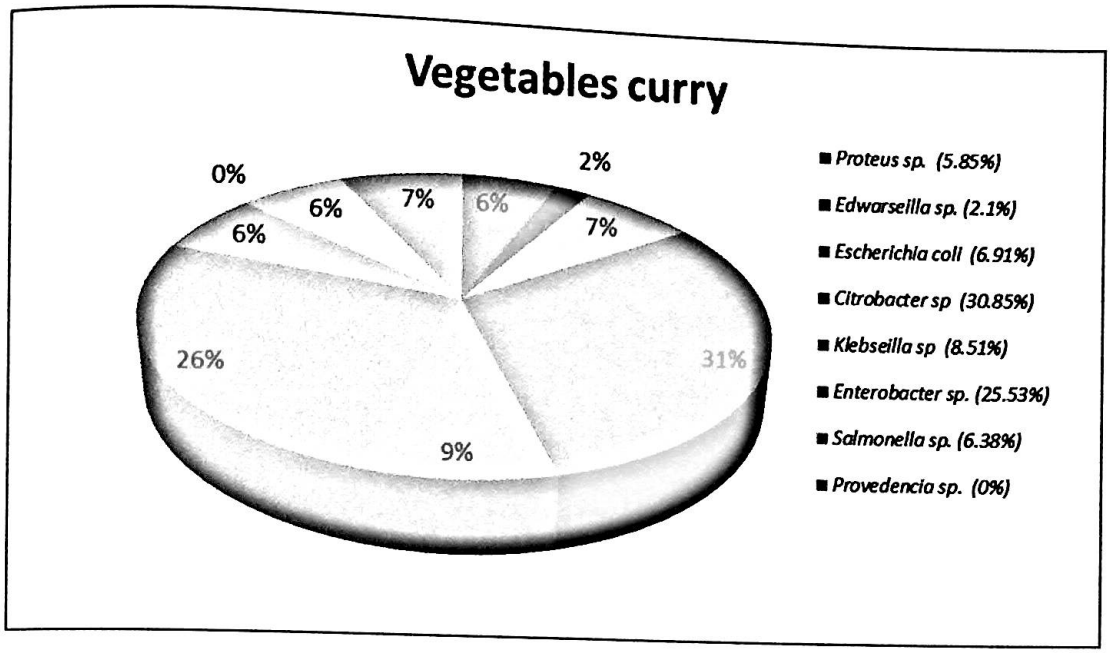

Fig 4.11. Abundance of different bacteria in vegetables curry.

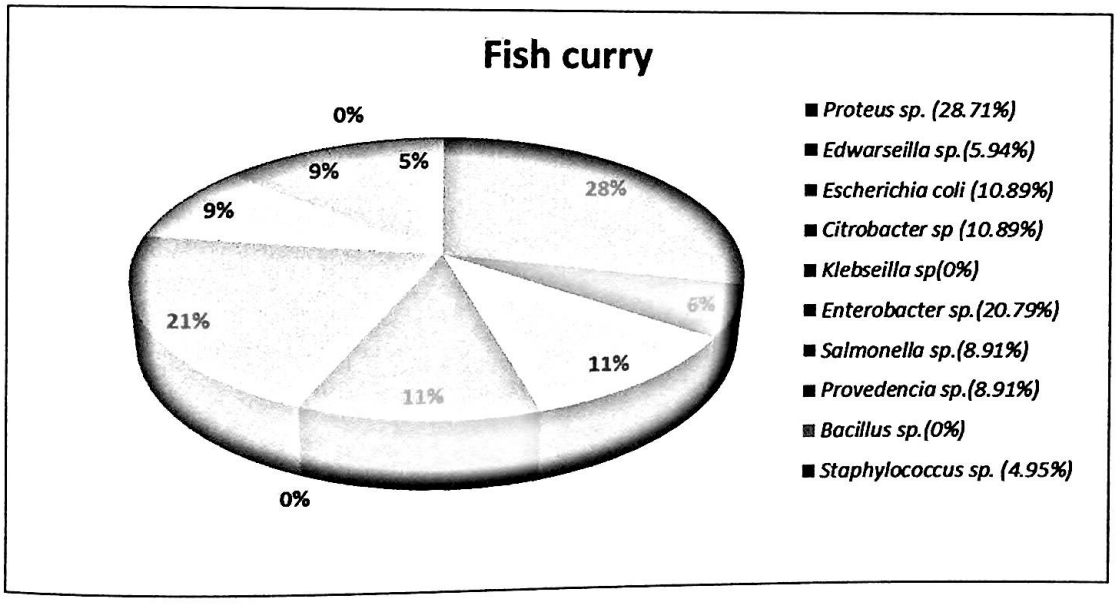

Fig 4.12. Abundance of different bacteria in fish curry.

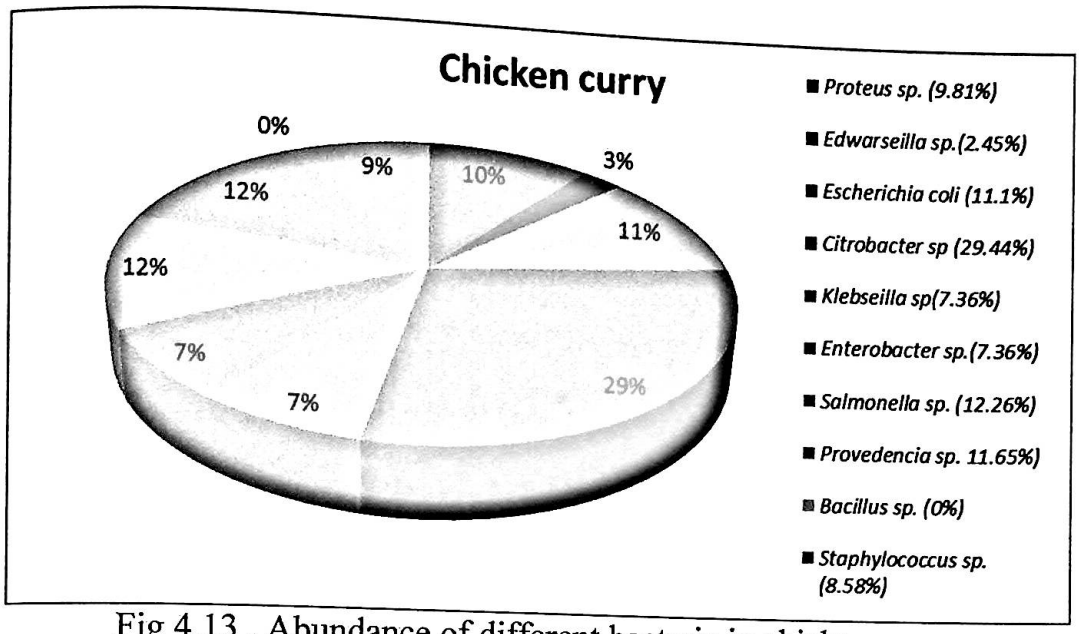

Fig 4.13 . Abundance of different bacteria in chicken curry.

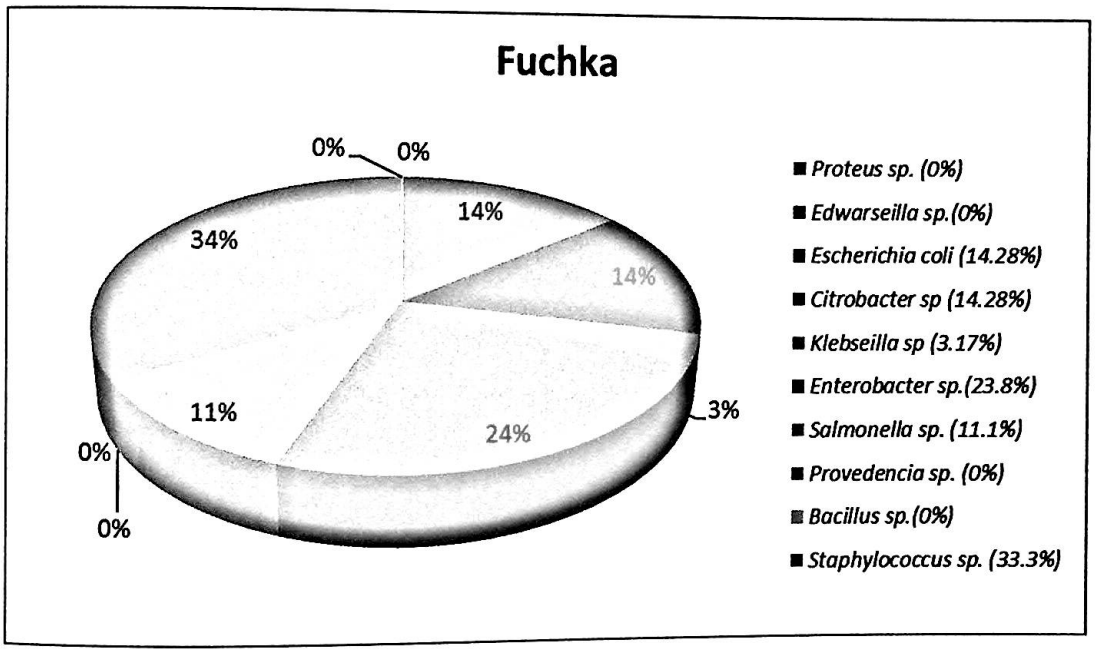

Fig 4.14. Abundance of different bacteria in fuchka.

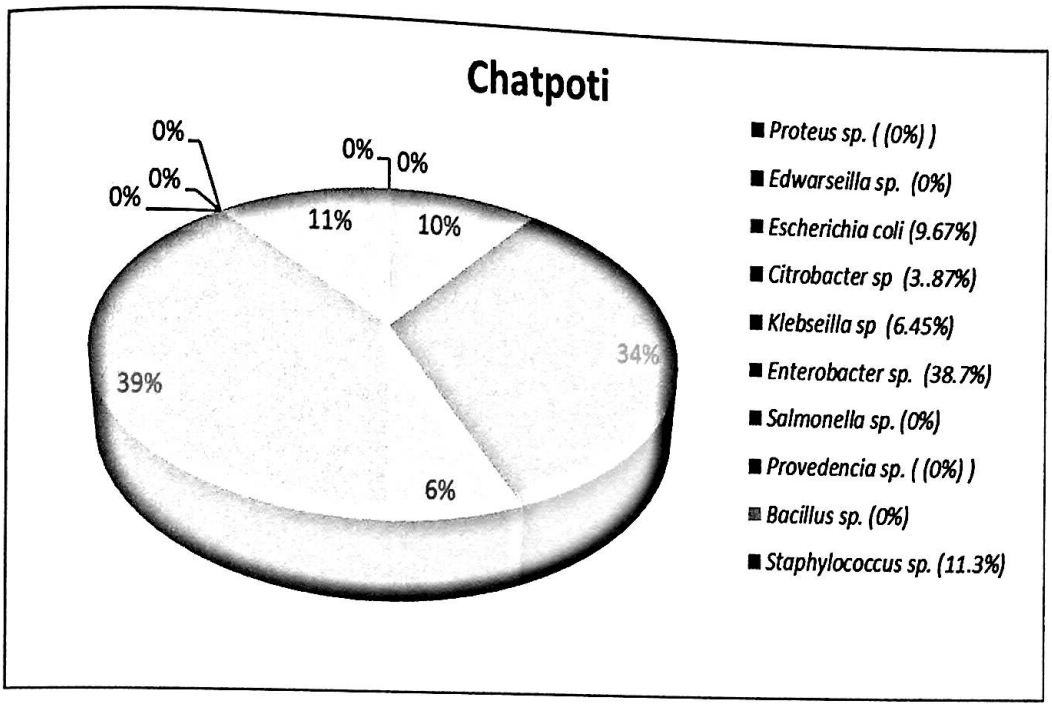

Fig 4.15 Abundance of different bacteria in chatpoti.

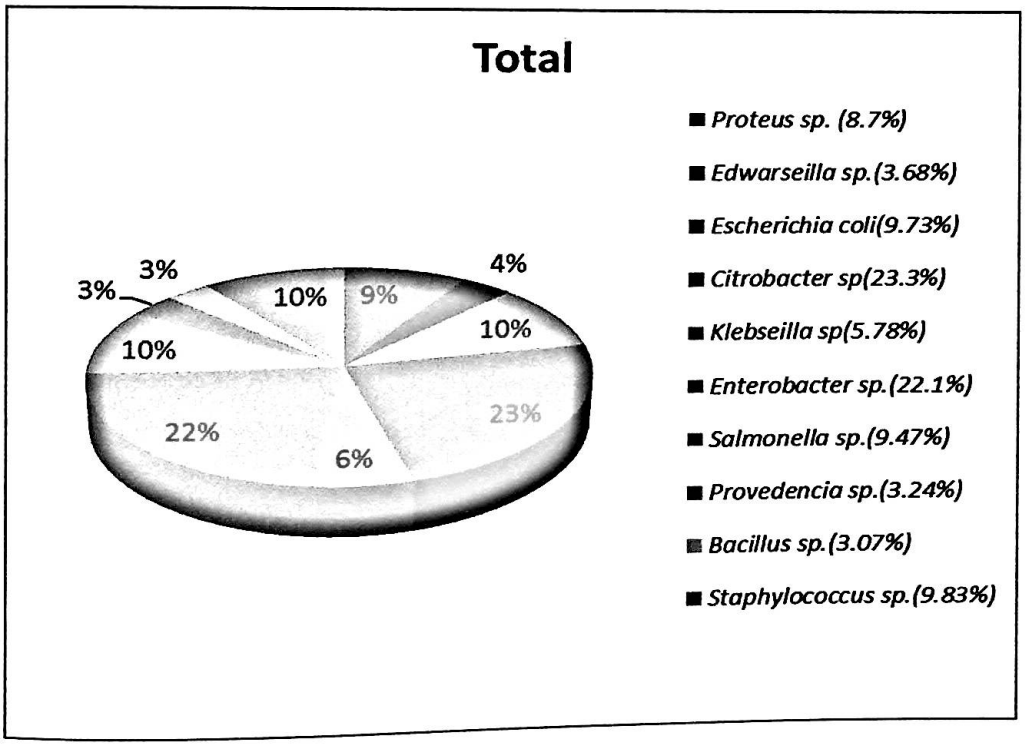

Fig 4.16 Total abundance of different bacteria in RTE food items sold in Rajshahi Metropolis.

4.5 Pathogenecity test of the selected isolates of different RTE foods.

The pathogenicity test was carried out by hemolytic activity test on blood agar medium, direct ingestion in albino mice (*Mus musculus*) and serological agglutination test.

4.5.1 Hemolytic activity test

Broth culture of the isolates were streaked with an inoculating loop on blood agar medium at 37^0C for 24 hours. Out of 62 isolates, nine isolates i.e. SMRS-651, RKFE-227, SMRS-671, BAFM-417, RSVE-185, BHVM-455, CMCN-243, RKRM-211 and LT DS-79 showed positive β-hemolytic activity (+++) and the results are presented in Table 4.27 and Plate 4.8. These isolates were selected for further experiment.

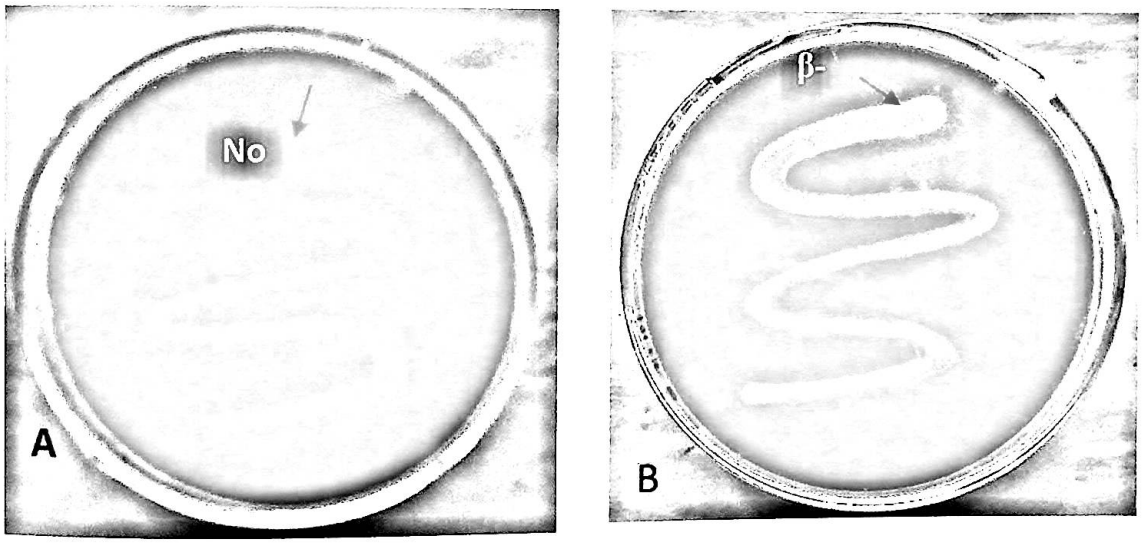

Plate 4.8. Photographs showing no hemolysis (A) & β-hemolysis (B) activity.

4.5.2 Virulence test results of the selected bacteria through direct ingestion of albino mice (*Mus musculusl*)

Nine β-hemolytic positive isolates were considered for virulence test on mice. Among the isolates, SMRS-671 (*Salmonella*), BAFM-417 (*Enterobacter*) and RSVE-185 (*E. coli*) no isolates showed highly toxic reactivity and the mice were died within 3 days and as well as SMRM-651 (*Proteus vulgaris*) and RKFE-227 (*Proveidencia*) no isolates ingested mice also died within 6 days. RKRM-211 (*Enterobacter*) no isolates showed severe illness symptom and rest of three isolates i.e. BHVM-455 (*Citrobacter*), CMCN-243 (*Enterobacter*) and LTDS-79 (*Citrobacter*) showed illness symptom after 12 days (Table 4.28 and Plate 4.9A&B). In control no toxic effect was found and the mice were remained healthy.

Table 4.28: Virulence test results of the selected bacteria on mice

Isolates Code	Constant dose 1.5×10^8	Initial Weight (gm)	Weight (gm) after				Average Weight Loss (%)	Symptoms observed
			3 days	6 days	9 days	12 days		
SMRM-651	+	28.43	23.37	-	-	-	-	Dead
RKFE-227	+	26.93	20.14	-	-	-	-	Dead
SMRS-671	+	30.68	-	-	-	-	-	Dead
BAFM-417	+	29.76	-	-	-	-	-	Dead
RSVE-185	+	27.37	-	-	-	-	-	Dead
BHVM-455	+	34.37	33.37	30.03	28.33	26.05	24.2	Illness
CMCN-243	+	35.93	32.14	31.99	29.6	28.4	20.95	Illness
RKRM-211	+	40.37	37.9	36.01	34.33	33.4	17.26	Severe Illness
LTDS-79	+	35.33	32.1	30.07	28.2	27.2	23.65	Illness
Control	-	26.20	29.07	30.71	32.11	33.65	28.43	Healthy

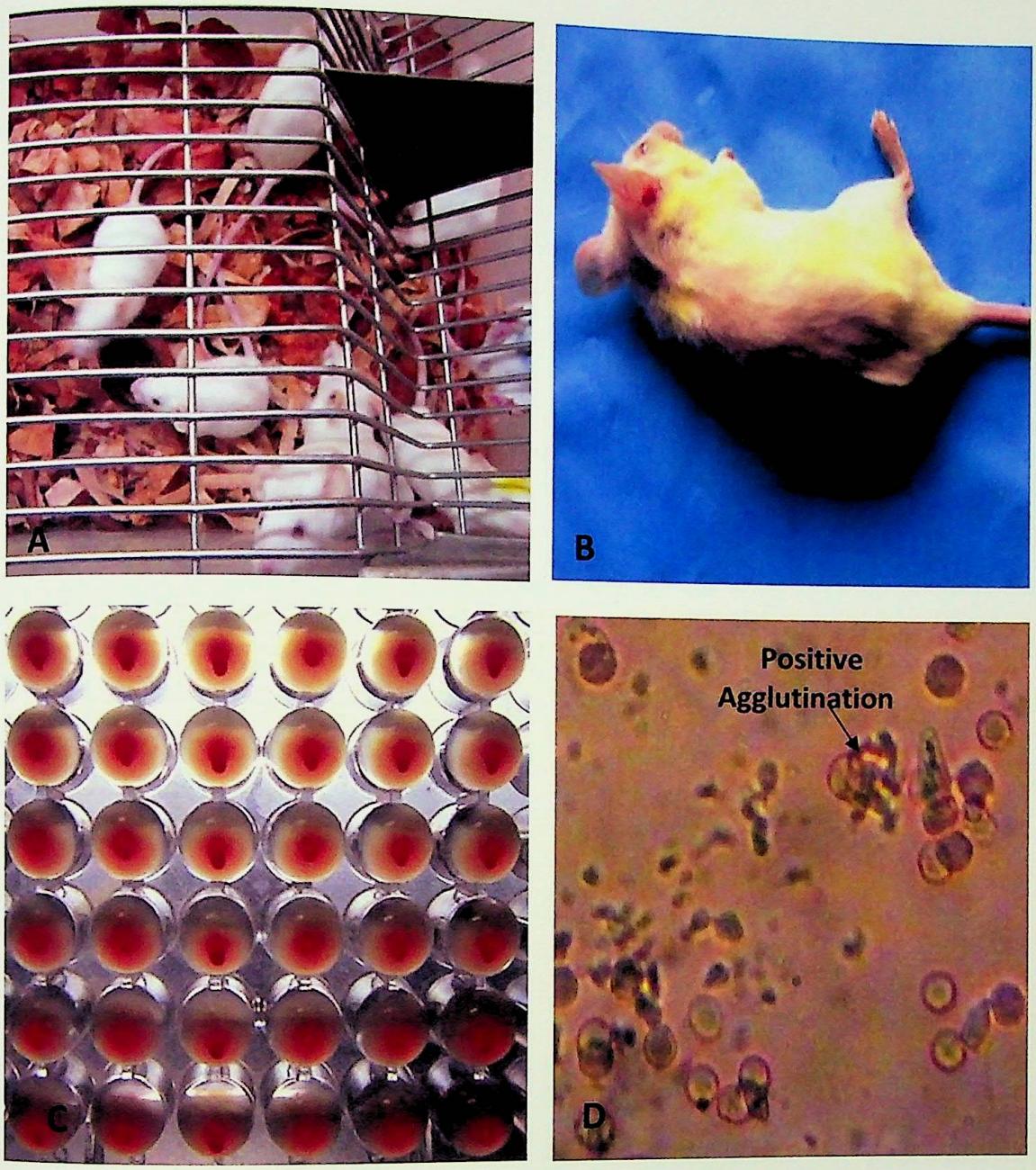

Plate 4.9. Photographs showing healthy albino mice (A), affected mice (dead) (B), gglutination occurred in micro-titer plate (C) and microscopic view of positive hemagglutination (D).

Chapter Five

Discussion

DISCUSSION

Ready to eat (RTE) foods are important sources of nutrition with economic importance. Now-a-days, these foods are being easily contaminated by the different micro-organisms and remain at alarming rate. In the present investigation, hygienic and sanitation status of RTE food selling restaurants and street food vans and personnel of six popular RTE food vending sites namely Laxmipur, Court, Naodapara, Saheb Bazar, RU Campus, Binodpur of Rajshahi Metropolis were surveyed during March 2015 to February 2016. Microbial quality of seven RTE food samples namely boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti were assesd by using different medium. Microbial diversity in different RTE food items were analysed by determine of frequency and abundance of the bacterial species. The pathogenicity test the isolates were carried out by hemolytic activity test on blood agar medium, direct ingestion in albino mice and serological agglutination test.

Survey on RTE food vendors and vending sites

RTE 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 RTE 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. 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.*). 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).

In the present study the survey were mainly conducted to ascertain the socioeconomic condition, general hygiene and sanitation practice of food seller as well as their waste management system to reduce the contamination rate and to protect public health. The sanitation condition of the restaurants of the surveyed areas was assessed. Most of the restaurants were clean regularly with normal detergent powder and sanitizer was not used. Waste management system of the restaurants of Saheb Bazaar were better while RU campus, Binodpur, Laxmipur were good and court and Naodapara areas were poor. In overall perception, it was observed that the 30% restaurants of Shaheb Bazar, 20% of RU campus, 15% of Binodpur, 7.6 % of Laxmipur were in best conditions and rest of the restaurants were

not good. From the survey data it was observed that all (100%) of the restaurant vendors were male. Age ranges of the majority (33.84%) of the vendors were 25-29 years and next of 32.31% vendors were 40-49 years old and 26.15% restaurants were below 25 years and only 7.69% were 50 plus. The maximum (42%) restaurant vendors were passed SSC, 29% were passed primary, 23% were passed H.S.C and only 6% restaurant vendors were illiterate. The majority (32.30%) of restaurants vendors earned 26000 to 30000 taka monthly and minimum (8% vendors) earned 10,000 to 15,000 taka. The most of the restaurant vendors (58%) had no knowledge about food hygiene. Most of them knew regular hand washing practices with soap and water (85%), proper covering of food (92.30%), storage of food in refrigerator (76.92%) and very much irregular for cleaning of sale surfaces (23.07%)). Hand washing before and after serving was practiced by (23.07%) respondents (30.77%) used aprons and none of respondents covered hair with caps or used gloves while serving food. They used tube-well and supply water for drinking and washing purpose. The utensils used in serving food were considered clean in majority (53.84%) cases and 100% of the restaurants used detergent for cleaning of utensil. There were 48% restaurant remained in poor sanitation condition while only 17% were in better condition. None of the respondents have any training about food safety and majority (92.30%) were not aware of food safety law. In case of street vendors, it was observed that majority (100%) of street vendors were male. Most of the restaurants vendors (60%) were 36-45 years, 20% vendors were 46-55 years, 12% were 26-35 years and 8% were below 15 years. For education level, most of the street vendors (60%) were passed JSC, 20% were passed primary, 12% were passed S.S.C and only 8% str[illegible]lors w[illegible] illiterate. The majority (52%) of street vendors earned 1 1[illegible]0 taka

monthly and minimum (12% vendors) earned 5,000 to 10000 taka. Most of the street food vendors (80%) had no knowledge about food hygiene. None of the vendors (100%) used apron, gloves while selling RTE food and did not covered food with polythene. In earlier study Gowned *et al.* (2013) studied socioeconomic profile of street food vendors in Allahabad City, India and reported that the street food sector is dominated by males, and 28.5% of them attended primary school and only 9% were graduate. 33.3% vendors washed their hands before and after the preparation of food. Bereda *et al.* (2016) studied street foods of Jigjiga City and reported that The majority of the street food vendors were women, 120(90.9%), with the average age group of 23–49 years (42.85%) and 99(66.7%) them were illiterate. Adjrah *et 89al.* (2013) surveyed street food in Lomé and reported that the street food sector is dominated by females, and 71.11% of them attended school. They lacked training on food hygienic and sanitary practice, and personal hygiene was not observed. Isara *et al.* (2017) assessed the food hygiene and safety practices of mobile food vendors in Benin City, Nigeria. There were 24 (9.6%) males and 226 (90.4%) females. Most of them knew regular hand washing practices with soap and water (95.5%), proper covering of stored food (92.8%), proper cleaning of cutting/sale surfaces (89.6%). Hand washing before and after serving food was practiced by 115 (46.0%) respondents, 14 (5.6%) used hand gloves while serving food, 143 (57.3%) properly covered their hair. Tonelada *et al.* (2018) investigated the food safety knowledge and practices of street food vendors of Tarlac State University and reported that majority of vendors are female (60%). Half of the total of the respondents had attained secondary education (50%). Majority of the respondents (80%) equally did not have any training on food safety and were not aware of food safety law. Nurudeen *et al.* (2014) reported that food venders of Nigeria lacked

basic training on hygiene and only 2.7% had formal training on food preparation. Abdalla *et al.* (2009) reported that food vendors of Atbara city were 28% male and 72% were female, 48% of them had primary school education while 42% were illiterates. The present findings support more or less of the above findings. From the survey report it may cocluded that RTE food sellers of Rajshahi Metropolis have few knowledge about food hygiene but their food safety practices were very poor.

Assessment of microbial quality of RTE foods

Microbial quality assessment is an integral part of any product development as it gives a clue to the safety and keeping quality of the food (Sankaran,1984). Total viable count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality. The presence of aerobic organisms reflects existence of favorable conditions for the multiplication of microorganisms. Microbial quality of seven types of RTE food items namely boiled rice, vegetable curry, cooked dal, fish curry, chicken curry, fuchka and chatpoti sold in Rajshahi Metropolis were assessed. From the results it was observed that the ranges of cfu total viable bacteria were recorded as 3.4×10^{6} to 6.2×10^{12}/gm in boiled rice, 1.24×10^{7} to 9×10^{11}/gm in vegetable curry, 3.4×10^{7} to 8.8×10^{12}/gm in chicken curry, 1.3×10^{6}/gm to 4.6×10/12 in cooked dal, 2.0×10^{7} to 6.5×10^{12}/gm in fish curry, 34.7×10^{5}/gm to 51.4×10^{10}/gm in chatpoti and 41.7×10^{6} to 52.8×10^{12}/gm in fuchka. The maximum cfu of boiled rice, vegetable curry, chicken curry, cooked dal and fish curry were recorded at Laxmipur and the minimum was recorded at Shaheb Bazar. Hundred percent samples contained $\geq 10^{6}$ cfu/gm which crosses the acceptable level of HACCP. In earlier study Henry *et al.* (2017) studied the microbiological quality of six RTE foods and reported that aerobic plate count for

stationary vendors with shade (SVWS) ranged from 1.20×10^6 to 4.20×10^6 cfu/g, for stationary vendors without shade (SVWOS), it ranged from 1.29×10^6 to 3.50×10^6 cfu/g, for mobile vendors (MV) ranged from 1.80×10^6 to 5.00×10^6 cfu/g. Okolie *et al.* (2012) investigated a total of sixty-four samples of four cooked foods from sixteen private primary schools in Akoka area of Yaba, Lagos, Nigeria and reported that the average microbial load for the rice, beans, meat and spaghetti samples are 3.5×10^4 cfu/ml, 6.9×10^4 cfu/ml, 4.9×10^4 cfu/ml, and 2.4×10^4 cfu/ml, respectively. Osakue *et al.* (2016) studied microbial quality of fried chicken samples in Benin city and reported that the mean values of bacteria count ranged from 4.67×10^6 to 1.2×10^7 cfu/g. The aerobic colony counts of bacteria were higher than International Food Standards ($<10^6$ cfu/g).

Coliform bacteria are one of the major indicators to detect hygienic level of RTE food. In this study large numbers of coliforms were recorded in boiled rice, vegetable curry, cooked dal, fish curry,chicken curry ,chatpoti and fuchka The ranges of total coliforms were recorded as 1.4×10^5 to 9.9×10^9/gm in boiled rice, 6.0×10^5 to 4×10^{10}/gm in vegetable curry, 1.1×10^6 to 5×10^{11}/gm in chicken and 8.4×10^6 to 4.8×10^{11}/gm in fish curry, 8.4×10^6 to 6.3×10^{10}/gm in cooked dal, $5.2\times10^3$to 8×10^9/gm in chatpoti and $2.1\times10^4$to 12.3×10^8 in fuchka. Hundred percent samples of boiled rice, vegetable curry, cooked dal, fish curry, chicken curry and 25-50% samples of chatpoti and 25-75% samples The maximum number of total coliforms were counted in chicken curry at Laxmipur and minimum was in vegetables curry at Saheb Bazaar. But in case of street food, chatpoti contained large number of coliforms at Saheb Bazaar with 75% of contamination while 100% samples of fuchka contained unacceptable level ($>10^4$ cfu/g) of coliforms at Naodapara. Fowoyo and Ali (2015) studied

microbial quality fried chicken, fried rice, meat pie and in Lokoja. The bacterial count of food samples ranged from 7.0×10^3 to 2.23×10^5 cfu/gm while coliform count ranged from 6.0×10^5 to 1.175×10^5 cfu/g. Cho *et al.* (2011) evaluated the microbial quality of street-vended foods in Korea and reported total coliform ranged from 0 to 6.86 cfu/g.

Further, *E. coli* and *Salmonella* sp. counted from ready to eat foods are indication of poor sanitary control and practices because they are known as food borne pathogen. The ranges of cfu of *E. coli* were recorded as 0 to 6.6×10^4 cfu/gm in boiled rice, 1×10^2 to 4×10^6/gm in vegetable curry, 1.12×10^2 to 5.2×10^5/gm in chicken curry, 0 to 9.4×10^4/gm in fish curry, 1.2×10^2 to 6.3×10^6/gm in cooked dal, 1.4×10^3 to 4.8×10^6/gm in chatpoti and 2.9×10^4 to 5.1×10^7/gm in fuchka. Hundred percent samples of boiled rice, chatpoti and fuchka , 17-100% percent samples of vegetable curry, 50-100% percent samples of chicken curry and cooked dal; 0-100% percent samples of fish curry were contaminated by $\geq 10^4$ cfu/gm of *E. coli*. Henry *et al.* (2017) studied the microbiological quality of six RTE foods and reported that *E. coli* count for stationary vendors with shade (SVWS) ranged from 0.9×10^6 to 2.50×10^6 cfu/g, for stationary vendors without shade (SVWOS), it ranged from 1.00×10^6 to 2.80×10^6 cfu/g and for mobile vendors (MV) ranged from 1.20×10^6 to 4.20×10^6 cfu/g.

Salmonella-Shigella were not recorded in boiled rice and vegetable curry but the ranges of cfu of Salmonella-*Shigella* were recorded as 0 to 6.8×10^2 cfu/gm in boiled rice, 0 to 2.8×10^2 cfu/gm, 0to 8.4×10^5/gm in chicken curry, 0 to 6.2×10^2/gm in fish curry, 0 to 9.2×10^4/gm in cooked dal, 0 to 5.1×10^3/gm in chatpoti, 5.1×10^2/gm in fuchka. 0 to 100% samples of boiled rice,0 to 50% samples of vegetable curry ,17-100% samples of chicken curry and 0- 50% percent samples of fish curry ,25-50% samples

of chatpoti and 25-100% samples of fuchka were contaminated by *Salmonella-Shigella.* by $\geq 10^1$ cfu/gm.

For *Staphylococcus* sp., counting of $\geq 10^6$cfu/g are considered potentially hazard for consumption of food. *Staphylococcus sp.* were not recorded in vegetable curry at Court and Naodapara area but the ranges of cfu of *Staphylococcus sp.* were recorded as 0 to 6.1×10^7/gm in boiled rice, 0 to 3.2×10^6/gm in vegetable curry,2×10^2 to 9.2×10^5/gm in chicken curry and 6×10^2 to 6.4×10^5/gm in fish curry, 2×10^3 to 3×10^6/gm in cooked dal, 2.5×10^6 to 9×10^8/gm in *Chapoti* and 4.4×10^4/gm to 7×10^8/gm in fuchka. 0 to 34% samples of boiled rice,0 to 34% samples of vegetable curry ,0% samples of chicken curry and fish curry , 50% samples of cooked dal, 25-50% samples of chatpoti and 50-100% samples of fuchka were contaminated by *Staphylococcus* were contaminated by $\geq 10^6$ cfu/gm. coliform. Henry *et al.* (2017) studied the microbiological quality of six ready-to-eat street foods and reported *Staphylococcus aureus* count at stationary vendors with shade (SVWS) ranged from 1.20×10^6 to 3.6×10^6 cfu/g , For stationary vendors without shade (SVWOS), it ranged from 1 1.60×10^6 to 4.00×10^6 cfu/g, for mobile vendors (MV) ranged from 1.60 $\times 10^6$ to 5.80×10^6 cfu/g.

Mean bacterial load of different RTE food items at different locations were analyzed. From the results it was observed that mean bacterial load varied with the bacterial species and sampling sites. In boiled rece the maximum mean cfu was counted as 12.27 $\log_{10}$ cfu/gm for TVC and followed by 8.96, 7.02 and 4.48 $\log_1$cfu/gm for coliform, *Staphylococcus* and *E. coli*, respectively. In vegetable The maximum mean cfu was counted for TVC (11.46 $\log_{10}$cfu/gm) and followed by 9.98, 6.41 and 5.80 $\log_{10}$cfu/gm for coliforms, *E. coli* and *Staphylococcus,* respectively. In

chicken curry the maximum mean cfu was counted for TVC (12.64 $\log_{10}$ cfu/gm) and followed by 10.94, 5.41 and 5.24 $\log_{10}$cfu/gm for coliforms, *Staphylococcus* and *E. coli,* respectively. In fish curry, the maximum mean of cfu was counted for TVC (12.32 $\log_{10}$ cfu/gm) and followed by 10.94, 5.30 and 4.36 $\log_{10}$cfu/gm for coliform, *staphylococcus* and *E. coli,* respectively. In cooked dal, the maximum mean cfu was counted for TVC (12.48 $\log_{10}$ cfu/gm) and followed by 10.26, 6.36 and 6.22 $\log_{10}$cfu/gm for coliform, *E. coli* and *Staphylococcus,* respectively. In chatpoti, t he maximum mean cfu was counted for TVC (11.11$\log_{10}$cfu/gm) and followed by 8.54, 8.15 and 6.35 $\log_{10}$cfu/gm for S*taphylocus,* coliform and *E. coli,* respectively. In fuchka, the maximum mean cfu was counted for TVC (13.12 $\log_{110}$cfu/gm) and followed by 8.68, 8.53 and 7.23 13.12 $\log_{110}$cfu/gm for coliform, *Staphylococcus* and *E. coli,* respectively. The minimum number of bacterial count was recorded for *Salmonella* (1.11-1.41 $\log_{10}$cfu/gm). The minimum cfu of *Salmonella* was reorded for in all cases. In earlier study, Chung *et al.* (2010) investigated the levels of total viable counts of RTE foods and showed a relatively wide range of cfu 1.9-7.8 $\log_{10}$ /gm. Nyenje *et al.* (2012) studied the microbiological quality of RTE food and observed the high levels of cfu of total aerobic count 6.8 ± 0.07 log /gm in rice, 6.7 ± 1.7 log /gm in vegetables and 6.27 ± 0.18 log /gm in chicken stew. Oranusi *et al.* (2013) investigated the microbiological quality RTE food and showed the ranges of cfu of coliform as 5.2×10^3 to 7.8×10^4 /gm. Similar findings were observed by Öz *et al.* (2014) who reported ≥10 cfu/gm to $\leq10^6$ cfu/gm *of E. coli* in RTE foods. Mashak *et al.* (2015) investigated the microbiological quality of RTE foods and recorded as 3.7 log cfu/gm of *Salmonella-Shigella.* Coniglio *et al.* (2016) investigated the microbiological quality of RTE foods and observed the *E. coli* contamination $<10^2$ cfu/gm. This finding

suggests that the street foods sold in Rajshahi Metropolis may be heavily contaminated with pathogenic microorganisms.

Characterizations and Identification of the isolated bacteria

Total 1140 isolates were isolated from RTE food samples of different restaurants and street vendors at six hot spots in Rajshahi Metropolis. For primary screening, Gram staining, catalase and motality test was performed. On the basis of morphological and microscopical characteristics, Gram staining, catalase and motality test the isolates were primarily grouped into sixty two and these 62 isolates were selected for further biochemical test. For confirmation of identification of the bacterial isolates, the results of biochemical tests had been inputted in online bacteria identification software ABIS & Micro Rao (Isolates of MS medium) which showed the percentages of conformity with the similarity of particular bacterial species. In boiled rice total eight isolates were tested and they were finally identified as *Bacillus tequilensis (*LERN-81) 95%, *Enterobacter aerogenes* (RKRM- 211) 99.9%, *Citrobacter kosery* (CKRE 301) 98%; *Proteus vulgarir* (SMRM-651) 98%; *Salmonella typhi (SMRS-67)* 96.8%, *Klebseilla oxytoca* (LCRE-22) 98%; *Edwardsiella tarda* (LERE-101) 99%; *Escherichia coli* (CMRE-261) 99%. In vegetable curry, total ten isolates were tested and they were finally identified as *Citrobacter frundii* (LCVN-5) 92.1%, *Klebseilla oxytoca* (LCVE-25) 99.97%, *Citrobacter kosery* (LTVN-45) 98%; *Escherichia coli* (RSVS-185) 99%, *Edwardsiella tarda* (CBVN-295) 99%, *Enterobacter aerogenes* (CBVN-325) 99.9%, *Salmonella typhi* (LCVS-35) 96.8%, *Bacillus tequilensis* (SMVN-645) 95%, *Proteus vulgaris* (BAVM-416) 98%; *Citrobacter kosery* (BHVM-455 98%. In cooked dal, total nine isolates were tested and they were finally identified as *Citrobacter*

(LCDM-19) with the conformity of 98%, *Enterobacter aerogene* (BRRN-379) 99.9% , *Klebseilla oxytoca (* BADE-4 29) 98%, *Escherichia coli* (RKDE-229) 99%, *Citrobacter koseri* (LTDN-49) 88%; *Citrobacter frundii* (LTDS-79) 90.4%, *Bacillus tequilensis* (BADN-409) 95%, *Proteus vulgarirs* (SMDM-659) 98%, *Salmonella typhi* (LEDS-120) 96.8%. In chicken curry, total eight isolates were tested and they were finally identified as *Proteus vulgarirs* (LCCM-13) 98%, *Salmonella typhi* (LCCS-33) 96.8%, *Esherichia coli* (LECE-103) 99%, *Citrobacter frundii* (RCCM-133) 92.01%, *Citrobacter koseri* (RCCS-153) 88%, *Enterobacter aerogene s(*CMCN-243) 89.9% , *Proveidencia alcalifaciens* (SACM-693) 99%, and *Klebseilla oxytoca* (SACS-713) 99.97%. In fish curry, total eight isolates were tested and they were finally identified as *Enterobacter aerogenes* (BAFS-438) 89.9%, *Proveidencia alcalifaciens* (RKFE-227) 99%, *Proteus mirabikis* (CKFE-297) 91.1%, *Enterobacter aerogenes* (BAFM-417) ,79.9%, *Edwardseilla tarda* (RKFE-218) 99%, *Citrobacter frundii* (CMFM-257) 92.1%, *Esherichia coli* (CBFE-348) 99% and *Salmonella typhi* (LCFS-37) 96.8%. In fuchka total five isolates were tested and they were finally identified as Enterobacter *aero genes* (CFuSS-754) 89.9%, *Klebseilla oxytoca (* BFuSS-796) 99.97%, *Esherichia coli* (RFuES-828) 99%, *Citrobacter kosery* (RFuSS-829) 88%, *Salmonella typhi* (CFuSS-766) 86.8%. In chatpoti, total five isolates were tested and they were finally identified as Enterobacter *aerogenes* (CChNS-772)-89.9%, *Klebseilla oxytoca* (LChES-747) -98%, *Citrobacter kosery* (LChMS-744) with the conformity of 88%, *Esherichia coli* (NChES-906) - 99%, *Citrobacter frundii* (SChSS-906) -97.4%. Only seven isolates isolated from MS medium were finally identified as *Staphylococcus aureus* with the conformity of 89.6% . Finally, the identified bacteria in different RTE food items were identified as ten genera i.e *Proteus,*

Edwardsiella, Klebsiella, Salmonella, Escherichia, Citrobacter, Enterbacter , Bacillus, Staphyloccous, Providencia. In earlier studies Rane (2011) reported that street foods to be microorganism belonging to the genus *Bacillus*, *Staphylococcus*, *Clostridium*, *Vibrio*, *Campylobacter*, *Listeria*, *Salmonella.* Oranusi *et al.* (2013) investigated the microbial quality of snacks (ready to eat foods) sold in Ota, Ogun State and Six different bacterial and three fungal isolates were identified as *E. coli, S. aureus, Bacillus cereus, Enterococcus, Klebsiella spp., Pseudomonas spp., Aspergillus niger, Penicillium sppd* . and Mucor. Das *et al.* (2010) studied the microbiological quality of street foods like panipuri and bhelpuri sold in many parts of Bangalore, India and *Streptococcus faecalis*, *Escherichia coli, Staphylococcus aureus, Bacillus* sp., *Klebsiella* sp. and *Pseudomonas* sp. were identified as prevelant genera. The present results also support the findings of Rane (2011), Oranusi *et al.* (2013) and Das *et al.* (2010).

Detection of bacterial diversity among the RTE food items

Total 570 RTE food samples namely boiled rice, cocked dal, vegetables curry, fish curry, chicken curry, fuchka and chatpoti of Rajshahi Metropolis were tested. These isolates were identified as ten genera i.e *Proteus* sp, *Edwarseilla* sp., *Escherichia coli, Citrobacter* sp., *Klebseilla* sp., *Enterobacter* sp., *Salmonella* sp., *Provedencia* sp., *Bacillus* sp. and *Staphylococcus* sp. The frequency and abundance of these genera were determined. For boiled rice, the maximum frequency was recorded for *Enterobacter* sp. (42.2%) and the lowest was *Edwarsilla* sp. (8.8%). *Provedencia* sp. was not detected. For cooked dal, the highest frequency (53.3%) was recorded for *Citrobacter* sp. and the lowest was Bacillus sp (3.3%). *Provedencia* sp. was not detected. For vegetable curry, [illegible] highest frequency (64.4%) was detected for of *Citrobacter* sp. and the

lowest was *Edwarsilla* sp. (4.4%). *Provedencia* sp. was absent in vegetables curry.For fish curry, the highest frequency (64.4%) was recorded for *Proteus* sp. and the lowest was *Staphylococcus* sp (11.11%). *Bacillus* sp. and *Klebseilla* sp. were not detected. For chicken curry, the highest frequency was recorded for *Citrobacter* sp. (53.3%) and the lowest was *Edwarseilla* sp.(4.4%). *Bacillus* sp not detected. For fuchka , the highest frequency was recorded for *Staphylococcus* sp. (70%) and the lowest was *Klebseilla* sp.(6.6%) . *Proteus* sp., *Edwarseilla* sp., *Bacillus* sp. and *Provedencia* sp. were not detected.For chatpoti, the highest frequency was detected for of *Enterobacter* sp. (80%) and the lowest was *Staphylococus* sp.(2.33). *Proteus* sp., *Edwarseilla* sp., *Salmonella* sp., *Bacillus* sp. and *Provedencia* sp. were not detected. In overall, it was observed that the most prevalent genera was *Ctrorobacter*(46.6%). Next of *Enterobacter* (44.38%), *Staphylococcus* (19.64%), *E.coli*(19.47%), *Salmonella* (18.94%),*Proteus (17*.54%), *Klebseilla* (11.57%) *Edwaeseilla* (7.36%), *Provedencia* (6.49%) and the lowest frequency was *Bacillus.*

In boiled rice the prevalence of the bacteria were 23.75% for *Enterobacter*, 18.75 for *Citrobacter* sp., 12.5% for *Bacillus* sp., 10% for *Proteus* sp. The most three dominant bacteria were *Enterobacter*, *Citrobacter* and *Bacillus* and which contributed 55% of the total bacteria in boiled rice. Cooked dal samples contained total nine genera and the prevalence of the bacteria were 27.11% for *Citrobacter*, 19.77% for *Enterobacter* sp., 18.1% for *Salmonella* sp., 9.03% for *E.coli*, 9.03 *Klebseilla* sp, *Edwarseilla* sp, 5.01% for *Proteus* sp, 3.38 % for *Staphylococcus* and 1.9% for *Bacillus* sp. The most three dominant bacteria were *Enterobacter, Citrobacter* and *Salmonella* which contributed 64.98% of the total bacteria in cooked dal. Vegetable curry samples contained total nine genera and The prevalence of the bacteria were

30.85% for *Citrobacter*, 25.53% for *Enterobacter* sp., 8.51% for *Klebseilla* sp, 7.44% for *Staphylococcus* sp, 6.91% for *E. coli*; 6.38% for *Salmonella* sp. and *Bacillus* sp. The most three dominant bacteria were *Enterobacter* sp, *Citrobacter* sp and *E. coli* which contributed 85.75% of the total bacteria in vegetable curry. Fish curry samples contained total eight genera of the bacter*ia.* The prevalence of the bacteria were 28.71% for *Proteus* sp, 20.79% for *Enterobacte*r sp., 10.79% for *E. coli* and *Citrobacter* 8.91% for *Salmonella* sp, 8.9% for *Proveidencia* sp, 5.94% for *Edwarseilla* sp, 4.95% for *Staphylococcus* sp. The most three dominant bacteria were *Proteus* sp, *Enterobacter* sp, and *Citrobacter* sp which contributed 60.38% of the total bacteria in fish curry . Chicken curry samples contained total nine genera and The prevalence of the bacteria were 29.44% for *Citrobacter* sp, 12.26% for *Salmonella* sp., 11.65% for *Provedencia* sp, 11.1% for *E. coli,* 9.81% for *Proteus* sp., 8.58% for *Stpphylococcus* sp; 7.36% for *Klebseilla and Enterobacter* sp; and 2.45% for *Edwarseilla* sp. The most three dominant bacteria were *Citrobacter* sp, *Salmonella* sp and *Provedencia* sp which contributed 53.35% of the total bacteria in chicken curry . Fuchka samples contained total six genera and The prevalence of the bacteria were 33.3% *for Staphylococcus* sp, 23.08% for *Enterobacte*r sp; 14.28% *E. coli* and *Citrobacte*r sp; and 11.1 % for *Salmonella* sp. and 3.17% for *Klebseilla* sp. The most three dominant bacteria were *Enteobacter* sp , *Staphylococus* sp *and Citrobacter* sp which contributed 71.38% of the total bacteria in Ffuchka. Chatpoti samples contained total five genera of the bacter*ia and* The prevalence of the bacteria were 38.7% for *Enteobacter* sp, 33.87% for C*itroboracte*r sp, 11.3% for Staphylococcus sp, 9.67% for *E. coli*, 6.45% f*Klebseilla* sp. The most three dominant bacteria were *Citrobacte*r sp, *Enteobacter* sp and *Staphyloccus* which contributed 83.87% of the to[illegible] In overall, *Citrobacter* sp was the most frequent genera [illegible] showed 23.[illegible] of total abundance. Next of most dominating bacteria w[illegible] *Enterobacte*r

(22.19%), *Salmonella* sp (9.47%), *Proteus* sp (8.7%), *Klebseilla* sp (5.78%), *Edwarseilla* sp (3.68%) and *Proveidencia* sp(3.24%). The least dominanting bacteria was *Bacillus* sp (3.07%). On the other hand, *Enterobacter* sp, *Citrobacter* sp, *E. coli* and *Staphylococcus* sp were commonly found in all types of RTE food items. *Salmonella* sp was absent in chatpoti, *Proteus* sp was absent in fuchka and chatpoti; *Klebsilla* sp was absent in fish curry; *Proveidencia* sp was absent in boiled rice, cooked dal, vegetable curry, fuchka and chatpoti; and *Bacillus* sp absent in fish curry, chicken curry, fuchka and chatpoti. In earlier study, Hosein *et al.* (2008) determined the prevalence and microbial load of *Listeria spp., Escherichia coli* O157 and *Salmonella spp.* in ready-to-eat products in supermarkets across Trinidad and reported that he prevalence of *L. monocytogenes* was 1.7 %. Sixteen (4.5%) of 153 samples yielded *E. coli* but all samples were negative for *Salmonella spp.* and *E. coli* O157. Adjrah *et al.* (2013) studied microbiological quality of ready-to-eat (RTE) vegetable salads in Lomé and reported that *Salmonella spp.* was not detected in any of the samples evaluated, but almost 25% of the samples were contaminated by *S. aureus*. The percentage of samples positive for indicator of food safety lack germs like total aerobic bacteria, total coliforms, thermo tolerant coliforms were 100, 100 and 37.68 respectively. Tambekar *et al.* (2008) analysed street vended foods in several places of India and the bacterial pathogens were identified as *P. aeruginosa* (39%), *E.coli* (21%), *S. aureus* (16%), *Salmonella sp.* (12%) and *Proteus sp.* (12%). The highest frequencies of occurrence of bacterial pathogens were *P. aeruginosa* in samosa (25%), *E. coli* in kachori (32%), *S. aureus* in kachori (27%), *Proteus sp.* in palakwada (45%) and 36% *Salmonella sp.* in samosa. Sandra *et al.* (2012) reported that Nasi ayam have the highest prevalence (100%) of *B. cereus* compared to nasi putih (76.2%) and nasi lemak (70.4%). Nasi briyani had the lowest prevalence (50%) of *B. cereus*. The frequencies of *B. thuringiensis* were found to be 10, 30 and

35.2 % in nasi putih and nasi ayam, nasi briyani and nasi lemak respectively. Shafizi *et al.* (2016) reported that cooked RTE food had 50 (45%) of frequency for *S. aureus* compared to raw RTE with only 56 (55%).

Pathogenecity test of the selected isolates of different RTE foods

The pathogenicity test was carried out by hemolytic activity test on blood agar medium, direct ingestion in albino mice and hem-agglutination test. Out of 62 isolates, nine isolates i.e. SMRS-651, RKFE-227, SMRS-671, BAFM-417, RSVE-185, BHVM-455, CMCN-243, RKRM-211 and LT DS-79 showed positive β-hemolytic activity (+++) . Pathogenicity test of the selected isolates were carried out by hemolytic activity test, direct ingestion in albino mice (*Mus musculus*) and serological agglutination test. For another test of virulence, constant dose of 1.5×10^8 cfu/ml (McFarland OD 0.5) of each pathogenic bacteria were directly ingested to mice separately. Out of 62 isolates, nine isolates namely *Salmonella* sp. (SMRS -671), *Enterobacter* sp. (BAFM-417), *E.coli* (RSVE-185), *Proveidencia* sp. (RKFE-227) and *Proteus vulgaris* (SMRM-651), *Enterobacter* sp (RKRM-211), *Citrobacter* sp (LTDS-79), *Citrobacter* sp (BHVM-455) showed positive β-hemolytic activity on blood agar because these isolates break down red blood cells and resulting is a clear zone surrounding the colony. Isenberg (1992) and Islam M Z (2008) also reported the similar finding.

These Nine β-hemolytic positive isolates were considered for virulence test on mice. Among the isolates, SMRS-671, BAFM-417 and RSVE-185 no isolates showed highly toxic reactivity and the mice were died within 3 days and as well as SMRM-651 and RKFE-227 no isolates ingested mice also died within 6 days and rest of the isolates showed severe to

mild illness symptom. All the isolates also showed positive results for hem-agglutination. In various studies pathogenic bacteria were identified as *Bacillus*, *Staphylococcus*, *Clostridium*, *Vibrio*, *Campylobacter*, *Listeria*, *Salmonella* (Rane, 2011). Neter *et al.* (1952), stelma et al (1987) Islam M. Z (2018) reported the similar findings.

Hemagglutination assay was performed using mice RBC. On the basis of virulence test results, five isolated bacteria namely *Salmonella* sp. (SMRS-671), *Enterobacter* (BAFM-417), *E. coli* (RSVE-185), *Proveidencia* (RKFE-227) and *Proteus vulgaris* (SMRM-651) were considered for hemagglutination test. All the isolates showed positive agglutination result Neter *et al.* (1952) reported the similar findings.

From the results it may be concluded that microbial quality of RTE foods were very poor and heavily contaminated with coliforms, *Staphylococcus and E. coli.* The most virulent pathogenic bacteria were *Salmonella* sp., *Enterobacter* sp., *E. coli*, *Proveidencia* sp. and *Proteus vulgaris*. *Thus* application of sound risk analysis policies is being advocated to provide a scientific base to the vendors of risk management option which may need to explore to ensure public health and safety.

Summary

SUMMARY

RTE food plays an important socioeconomic role in meeting food and nutritional requirements of city consumers at affordable prices to the lower and middle income people. But the food safety problems are particularly becoming an increasingly serious threat to the public. In the present investigation, hygienic and sanitation status of RTE food selling restaurants and street food vans and personnel of six popular RTE food vending sites of Rajshahi Metropolis were surveyed during March 2015 to February 2016. Microbial quality and microbial diversity RTE foods samples namely boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti were assesd. The pathogenicity test of the isolates were carried out by hemolytic activity test, direct ingestion in albino mice and hemagglutination test.

Survey on RTE food vendors and vending sites

In the present study the survey were mainly conducted to ascertain the socioeconomic condition, general hygiene and sanitation practice of food seller as well as their waste management system to reduce the contamination rate and to protect public health. The sanitation condition of the restaurants of the surveyed areas was assessed. Most of the restaurants were clean regularly with normal detergent powder and sanitizer was not used. Waste management system of the restaurants of Saheb Bazaar were better while RU campus, Binodpur, Laxmipur were good and court and Naodapara areas were poor. In overall perception, it was observed that the 30% restaurants of Shaheb Bazar, 20% of RU campus, 15% of Binodpur,

7.6 % of Laxmipur were in best conditions and rest of the restaurants were not good.

From the survey data it was observed that all (100%) of the restaurant vendors were male. Age ranges of the majority (33.84%) of the vendors were 25-29 years. The maximum (42%) restaurant vendors were passed SSC, 29% were passed primary, 23% were passed H.S.C and only 6% restaurant vendors were illiterate. The majority (32.30%) of restaurants vendors earned 26000 to 30000 taka monthly. The most of the restaurant vendors (58%) had no knowledge about food hygiene. Most of them knew regular hand washing practices with soap and water (85%), proper covering of food (92.30%), storage of food in refrigerator (76.92%) and very much irregular for cleaning of sale surfaces (23.07%)). Hand washing before and after serving was practiced by (23.07%) respondents, 30.77% used aprons and none of respondents covered hair with caps or used gloves while serving food. They used tube-well and supply water for drinking and washing purpose. The utensils used in serving food were considered clean in majority (53.84%) cases and 100% of the restaurants used detergent for cleaning of utensil. There were 48% restaurant remained in poor sanitation condition while only 17% were in better condition. None of the respondents have any training about food safety and majority (85%) were not aware of food safety law.

In case of street vendors, it was observed that majority (100%) of street vendors were male. Most of the restaurants vendors (60%) were 36-45 years. Most of the restaurant vendors (64%) were passed JSC and only 6% street vendors were illiterate. The majority (52%) of street vendors earned 11000 to 15000 taka monthly Most of the street food vendors (80%) had no knowledge about food hygiene. None of the vendors (100%) used

apron, gloves while selling RTE food and did not covered food with polythene.

Microbial quality assessment of RTE foods

In the present study different RTE food samples were collected from restaurants and street food vendors of six hot spots of Rajshahi Metropolis and surrounding areas during March 2015 to February 2016. These food samples were subjected for microbial quality assessment. For this purpose Nutrient agar medium was used to total viable count (TVC), MacConkey agar (MA) was used for total coliform count, eosin metheline blue (EMB) was used for total *E. coli* count, *Salmonella-Shigella* (SS) agar medium was used for total *Salmonella* count and Manitol salt (MS) agar medium used for total *Staphylococcus*. Seven RTE food items namely boiled rice, vegetable curry, chicken curry, fish curry, cooked dal, fuchka and chatpoti were selected for TVC count. In boiled rice, the colony forming unit (cfu) of total viable bacteria were the ranged as 3.4×10^{6} to 6.2×10^{12} cfu/gm . The ranges of cfu were counted as 1.24×10^{7} to 9×10^{11} cfu/gm for vegetable curry. The ranges of cfu Chicken curry were recorded as 3.4×10^{7} to 8.8×10^{12} cfu/gm. samples were placed on nutrient agar (NA) medium and the colony forming unit (cfu) of total viable bacteria was counted. The ranges of cfu of fish curry were recorded as 2.0×10^{7} to 6.5×10^{12} cfu/gm. The ranges of cfu of cooked dal were recorded as 1.3×10^{6} to 4.6×10^{12} cfu/gm. Chatpoti samples contained total 34.7×10^{5} to 51.4×10^{10} cfu/gm and Fuchka contained total viable bacteria as 41.7×10^{6} to 52.8×10^{12}cfu/gm. Hundred percent samples of all types of RTE food contained $\geq10^{6}$ cfu/g.

In boiled rice, the ranges of cfu of total coliforms were recorded as 1.42×10^{5} to 9.9×10^{9} cfu/gm. Coliform contamination was detected in about hundred percent of the samples with $\geq10^{4}$ cfu/gm. In vegetable curry

the ranges of total coliforms were recorded as 6×10^5 to 4×10^{10} cfu/gm and Hundred percent samples were contaminated by total coliforms with $\geq10^4$ cfu/gm. In chicken curry the ranges of total coliforms were recorded as 1.1×10^6 to 5×10^{11} cfu/gm and hundred percent samples were contaminated by coliforms with $\geq10^4$ cfu/gm. In fish curry, the ranges of total coliforms were recorded as $8.4\times10^6$to 4.8×10^{11} cfu/gm and hundred percent of the samples contaminated with $\geq10^4$ cfu/gm. In cooked dal, MacConkey medium was used for total coliforms count and the ranges of total coliforms were recorded as 8.4×10^6 to 6.3×10^{10}cfu/gm and hundred percent of samples were contaminated with $\geq10^4$ cfu/gm. In chatpoti, the ranges of total coliforms were recorded as 5.2×10^3 to 8×10^6 cfu/gm and 25-50% of the samples contained $\geq10^4$ cfu/gm. In fuchka, the ranges of total coliforms were recorded as 2.1×10^4 to 12.3×10^8 cfu/gm and 25-75% of samples contained $\geq10^4$ cfu/gm.

In boiled rice, The ranges of cfu of total *E. coli* were recorded as 0 to 5.2×10^3 cfu/gm. 17-100% samples were contaminated by *E. coli* with $\geq10^2$ cfu/gm. In vegetable curry, the ranges of cfu of total *E. coli* were recorded as 1×10^2 to 4×10^6 cfu/gm while 17-100% samples were contaminated by *E. coli* with $\geq10^2$cfu/gm. In chicken curry, the ranges of cfu of total *E. coli* were recorded as 1.12×10^2 to 5.2×10^5 cfu/gm and 50-100% samples were contaminated with $\geq10^2$ cfu/gm. In fish curry the ranges of cfu of total *E. coli* were recorded as 0 to 9.4×10^4 cfu/gm and 0-100% samples were contaminated with $\geq10^2$ cfu/gm. In cooked dal, the ranges of cfu of total *E. coli* were recorded as 0 to 6.3×10^6 cfu/gm and 0-100% samples were contaminated by *E. coli* with $\geq10^2$ cfu/gm. In chatpoti, the ranges of total *E. coli* were recorded as 1.4×10^3 to 4.8×10^6 cfu/gm and hundred percent samples were contaminated by *E. coli* with $\geq10^2$ cfu/gm. In fuchka, the

ranges of *E. coli* were recorded as 2.9×10^4 to 5.1×10^7 cfu/gm and hundred percent samples were contaminated by *E. coli* with $\geq 10^2$ cfu/gm.

In boiled rice, SS medium was used for total *Salmonella* count and the ranges of cfu of total *Salmonella* were 0 to 6.8×10^2 cfu/gm. 0 to 100% percent samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm. In vegetable curry, SS medium was used for total *Salmonella* count and the ranges of cfu of total *Salmonella* were 0 to 2.8×10^2 cfu/gm. 0 to 84% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm. In fish curry, SS medium was used for total *Salmonella* count and the ranges of cfu of total *Salmonella* were counted as 0 to 6.2×10^2 cfu/gm. 0 - 50% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm. In chicken curry, SS medium was used for total *Salmonella* sp. count and the ranges of cfu of total *Salmonella* were counted as 0 to 8.4×10^5 cfu/gm. 17-100% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm. In cooked dal, SS medium was used for total *Salmonella* sp. count and the ranges of cfu of total *Salmonella* were counted as 0 to 9.2×10^4 cfu/gm. 17-100% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm. In fuchka, SS medium was used for total *Salmonella* sp. count and the ranges of cfu of total *Salmonella* were counted as 0 to 5.1×10^3 cfu/gm. 25 -50 % samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm. In chatpoti, SS medium was used for total *Salmonella* sp. count and the ranges of cfu of total *Salmonella* were counted as 0 to 5.1×10^2 , 25 -100% samples were contaminated by *Salmonella* with $\geq 10^1$ cfu/gm.

In boiled rice the ranges of cfu of total *Staphylococcus* were recorded as 0 to 6.1×10^7 cfu/gm. 0 to 34% samples were contaminated by *Staphylococcus* with $\geq 10^6$ cfu/gm. In vegetable curry, the ranges of cfu were recorded as 0 to 3.2×10^6 cfu/gm. 0 to 34% samples were

contaminated by *Staphylococcus* with $\geq 10^6$ cfu/gm. In chicken curry, the ranges of cfu of total *Staphylococcus* were recorded as 2×10^2 to 9.2×10^5 cfu/gm. No samples were contaminated with $\geq 10^6$ cfu/gm. In fish curry, the ranges of cfu of total *Staphylococcus* were recorded as 6×10^2 to 6.4×10^5 cfu/gm. No samples were contaminated with $\geq 10^6$ cfu/gm. In cooked dal, the ranges of cfu of total *Staphylococcus* were recorded as 2×10^3 to 3×10^6 cfu/gm. 0 to 50% samples were contaminated by *Staphylococcus* with $\geq 10^6$ cfu/gm. In chatpoti, the ranges of cfu of total *Staphylococcus* were recorded as 2.5×10^3 to 9×10^7 cfu/gm. 25-100% samples were contaminated by *Staphylococcus* with $\geq 10^6$ cfu/gm. In fuchka, the ranges of cfu of total *Staphylococcus* were recorded as 4.4×10^4 to 6.0×10^9 cfu/gm. 50-100% samples were contaminated by *Staphylococcus* with $\geq 10^6$ cfu/gm.

Mean bacterial load of different RTE food items at different locations were analyzed. In case of boiled rice, it was observed that mean bacterial load varied with the bacterial species and sampling sites. The maximum cfu was counted as 12.27 $\log_{10}$ cfu/gm for TVC and followed by 8.96, 7.02 and 4.48 $\log_1$cfu/gm for coliform, *Staphylococcus* and *E. coli*, respectively. The minimum number of bacterial count was recorded for *Salmonella* (0-3.83 $\log_{10}$ cfu/gm). The order of mean bacterial count for different types of bacteria in boiled rice were recorded as TVC> coliforms> *Staphylococcus* > *E. coli* > *Salmonella.*

In vegetable curry, it was observed that mean bacterial load varied with the bacterial species and sampling sites. The maximum mean cfu was counted for TVC (11.46 $\log_{10}$cfu/gm) and followed by 9.98, 6.41 and 5.80 $\log_{10}$cfu/gm for coliforms, *E. coli* and *Staphylococcus,* respectively. The minimum number of bacterial count was recorded for *Salmonella (0-2.2* $\log_{10}$ cfu/gm). The order of mean bacterial count for different types of

bacteria in vegetable curry were recorded as TVC> coliforms> *E. coli*> *Staphylococcus*> *Salmonella.*

In chicken curry, it was observed that mean bacterial load varied among the bacterial types and sampling sites. The maximum mean cfu was counted for TVC (12.64 $\log_{10}$ cfu/gm) and followed by 10.94, 5.41 and 5.24 $\log_{10}$cfu/gm for coliforms, *Staphylococcus* and *E. coli,* respectively. The minimum bacterial count was recorded for *Salmonella* (1.29 - 5.17 $\log_{10}$ cfu/gm). The order of mean bacterial count for different types of bacteria in vegetable curry were recorded as TVC > coliforms > *Staphylococcus* > *E. coli* > *Salmonella.*

In fish curry, it was observed that mean bacterial load varied among the bacterial types and sampling sites. The maximum mean of cfu was counted for TVC (12.32 $\log_{10}$ cfu/gm) and followed by 10.94, 5.30 and 4.36 $\log_{10}$cfu/gm for coliform, *staphylococcus* and *E. coli,* respectively. The minimum bacterial number was recorded for *Salmonalla* (0.52-3.36 $\log_{10}$cfu/gm). The order of mean bacterial count for different types of bacteria in vegetable curry were recorded as TVC > coliforms > *Staphylococcus* > *E .coli* > *Salmonella.*

In cooked dal mean bacterial load varied with the bacterial types and sampling sites. The maximum mean cfu was counted for TVC (12.48 $\log_{10}$ cfu/gm) and followed by 10.26, 6.36 and 6.22 $\log_{10}$cfu/gm for coliform, *E. coli* and *Staphylococcus,* respectively. The minimum number of bacterial count was recorded for *Salmonella* (1.83-4.17 $\log_{10}$cfu/gm).The order of mean bacterial count for different types of bacteria in vegetable curry were recorded as TVC > coliform > *Staphylococcus* > *E. coli* > *Salmonella.*

In chatpoti, it was observed that mean bacterial load varied with the bacterial types and sampling sites. The maximum mean cfu was counted for TVC (11.11$\log_{10}$cfu/gm) and followed by 8.54, 8.15 and 6.35 $\log_{10}$cfu/gm for S*taphylocus,* coliform and *E. coli,* respectively. The minimum number of bacterial count was recorded for *Salmonella* (1.11 - 2.41 $\log_{10}$cfu/gm). The order of mean bacterial count for different types of bacteria in chatpoti were recorted as TVC > *Staphylococcus* > *Coliform* > *E .coli* > *Salmonella.*

In fuchka, it was observed that mean bacterial load varied with the bacterial types and sampling sites. The maximum mean cfu was counted for TVC (13.12 $\log_{110}$cfu/gm) and followed by 8.68, 8.53 and 7.23 13.12 $\log_{110}$cfu/gm for coliform, *Staphylococcus* and *E. coli*, respectively. The minimum number of bacterial count was recorded for *Salmonella* (1.11-1.41 $\log_{10}$cfu/gm). The order of mean bacterial count for different types of bacteria in fuchka were recorded as TVC > *coliform* > *Staphylococcus* > *E. coli* > *Salmonella.*

On the otherhand, in boild rice, among the locations, the maximum number of TVC, *Staphylococus, coliforms* and *E. coli* were recorded at Laxmipur of and *Salmonella* at R.U campus. In vegetable curry, the maximum number of TVC, coliforms, *E. coli* and *Staphylococus* were counted at Laxmipur; and *Staphylococus* and *Salmonella* were not detected at Court and Naodapara areas. In chicken curry the maximum number of TVC, coliforms, *Staphylococcus, E. coli* and *Salmonella* were recorded at Laxmipur and minimum at Shaheb bazaar. In fish curry the maximum number of TVC, coliforms, *E. coli*, *Salmonella* were recorded at Laxmipur and *Staphylococus* at Naodapara. In cooked dal the [illegible]um TVC, coliform, *E. coli, Salmonella* and *Staphyloc*[illegible]

Laxmipur. In chatpoti , the maximum TVC, *E. coli, Salmonella* and *Staphylococcus* was recorded at Laxmipur and *coliform* was recorded at R.U campus. In fuchka, the maximum TVC, coliform *and E. coli* were recorded at Shaheb bazaar area but the maximum number of *Salmonella* sp. was recorded at Laxmipur ; and maximum number of *Staphylococus* was recorded at R.U campus and at Court area.

Isolation and Identification of the bacterial isolates of different RTE foods

For isolation of bacterial isolates, seven types of RTE food samples were plated on five media i.e. NA MacConkey agar, EMB agar , SS agar and MS agar medium. After 24 hours, bacterial colonies were visible on the surface of NA, MacConkey, EMB, SS and MS agar plates and the morphologically distinct colonies were identification and coded with their sources. The colony morphology of the isolates on nutrient agar, MacConkey agar, EMB agar and SS agar were observed according to form, color, elevation, margin. Total 1140 isolates were isolated. On the basis of Gram staining. motality and catalase test results these isolates were grouped into 62. Then the isolates are selected for biochemical test according to Bergy's Manuals. For confirmation of identification of the bacterial isolates, the results of biochemical tests had been inputted in online bacteria identification software ABIS (Isolates of NA, MacConkey, EMB andm SS medium) & Micro Rao (Isolates of MS medium) which showed the percentages of conformity with the similarity of particular bacterial species. Finally on the basis of morphology and microscopic characteristics, biochemical test results the isolates were identified and conformity percentages were recorded for each isolates.

In boiled rice total eight isolates were tested and they were finally identified as *Bacillus tequilensis* (LERN-81) 95%, *Enterobacter aerogenes* (RKRM- 211) 99.9%, *Citrobacter kosery* (CKRE 301) 98%; *Proteus vulgarir* (SMRM-651) 98%; *Salmonella typhi* (SMRS-67) 96.8%, *Klebseilla oxytoca* (LCRE-22) 98%; *Edwardsiella tarda* (LERE-101) 99%; *Escherichia coli* (CMRE-261) 99%. In vegetable curry, total ten isolates were tested and they were finally identified as *Citrobacter frundii* (LCVN-5) 92.1%, *Klebseilla oxytoca* (LCVE-25) 99.97%, *Citrobacter kosery* (LTVN-45) 98%; *Escherichia coli* (RSVS-185) 99%, *Edwardsiella tarda* (CBVN-295) 99%, *Enterobacter aerogenes* (CBVN-325) 99.9%, *Salmonella typhi* (LCVS-35) 96.8%, *Bacillus tequilensis* (SMVN-645) 95%, *Proteus vulgaris* (BAVM-416) 98%; *Citrobacter kosery* (BHVM-455 98%. In cooked dal, total nine isolates were tested and they were finally identified as *Citrobacter kosewri* (LCDM-19) with the conformity of 98%, *Enterobacter aerogene* (BRRN-379) 99.9% , *Klebseilla oxytoca* (BADE-4 29) 98%, *Escherichia coli* (RKDE-229) 99%, *Citrobacter koseri* (LTDN-49) 88%; *Citrobacter frundii* (LTDS-79) 90.4%, *Bacillus tequilensis* (BADN-409) 95%, *Proteus vulgarir*s (SMDM-659) 98%, *Salmonella typhi* (LEDS-120) 96.8%. In chicken curry, total eight isolates were tested and they were finally identified as *Proteus vulgarir*s (LCCM-13) 98%, *Salmonella typhi* (LCCS-33) 96.8%, *Esherichia coli* (LECE-103) 99%, *Citrobacter frundii* (RCCM-133) 92.01%, *Citrobacter koseri* (RCCS-153) 88%, *Enterobacter aerogenes* (CMCN-243) 89.9%, *Proveidencia alcalifaciens* (SACM-693) 99%, and *Klebseilla oxytoca* (SACS-713) 99.97%. In fish curry, total eight isolates were tested and they were finally identified as *Enterobacter aerogenes* (BAFS-438) 89.9%, *Proveidencia alcalifaciens* (RKFE-227) 99%, *Proteus mirabikis* (CKFE-297) 91.1%, *Enterobacter aerogenes* (BAFM-417) ,79.9%, *Edwardseilla*

tarda (RKFE-218) 99%, *Citrobacter frundii* (CMFM-257) 92.1%, *Esherichia coli* (CBFE-348) 99% and *Salmonella typhi* (LCFS-37) 96.8%. In fuchka total five isolates were tested and they were finally identified as *Enterobacter aerogenes* (CFuSS-754) 89.9%, *Klebseilla oxytoca* (BFuSS-796) 99.97%, *Esherichia coli* (RFuES-828) 99%, *Citrobacter kosery* (RFuSS-829) 88%, *Salmonella typhi* (CFuSS-766) 86.8%. In chatpoti, total five isolates were tested and they were finally identified as *Enterobacter aerogenes* (CChNS-772)-89.9%, *Klebseilla oxytoca* (LChES-747)-98%, *Citrobacter kosery* (LChMS-744)-88%, *Esherichia coli* (NChES-906)-99%, *Citrobacter frundii* (SChSS-906)-97.4%. Only seven isolates isolated from MS medium were finally identified as *Staphylococcus aureus* with the conformity of 89.6%. Finally, the identified bacteria in different RTE food items were identified as ten genera i.e *Proteus, Edwardsiella, Klebsiella, Salmonella, Escherichia, Citrobacter, Enterbacter, Bacillus, Staphyloccous, Providencia.*

Detection of bacterial diversity among the RTE food items

Total 570 RTE food samples namely boiled rice, cocked dal, vegetables curry, fish curry, chicken curry, fuchka and chatpoti of Rajshahi Metropolis were tested. These isolates were identified as ten genera i.e *Proteus* sp, *Edwarseilla* sp., *Escherichia coli, Citrobacter* sp., *Klebseilla* sp., *Enterobacter* sp., *Salmonella* sp., *Provedencia* sp., *Bacillus* sp. and *Staphylococcus* sp. The frequency and abundance of these genera were determined.

For boiled rice, the maximum frequency was recorded for *Enterobacter* sp. (42.2%) and the lowest was *Edwarsilla* sp.(8.8%). *Provedencia* sp.

was not detected.For cooked dal, the highest frequency (53.3%) was recorded for *Citrobacter* sp. and the lowest was *Bacillus* sp (3.3%). *Provedencia* sp. was not detected. For vegetable curry, the highest frequency (64.4%) was detected for of *Citrobacter* sp. and the lowest was *Edwarsilla* sp. (4.4%). *Provedencia* sp. was absent in vegetables curry.

For fish curry, the highest frequency (64.4%) was recorded for *Proteus* sp. and the lowest was *Staphylococcus* sp (11.11%). *Bacillus* sp. and *Klebseilla* sp. were not detected. For chicken curry, the highest frequency was recorded for *Citrobacter* sp. (53.3%) and the lowest was *Edwarseilla* sp.(4.4%). *Bacillus* sp not detected. For fuchka , the highest frequency was recorded for *Staphylococcus* sp. (70%) and the lowest was *Klebseilla* sp.(6.6%) . *Proteus* sp., *Edwarseilla* sp., *Bacillus* sp. and *Provedencia* sp. were not detected.For chatpoti, the highest frequency was detected for of *Enterobacter* sp. (80%) and the lowest was *Staphylococus* sp.(2.33). *Proteus* sp., *Edwarseilla* sp., *Salmonella* sp., *Bacillus* sp. and *Provedencia* sp. were not detected. In overall, it was observed that the most prevalent genera was *Ctrorobacter*(46.6%). Next of *Enterobacter* (44.38%), *Staphylococcus* (19.64%), *E.coli*(19.47%), *Salmonella* (18.94%),*Proteus (17*.54%), *Klebseilla* (11.57%) *Edwaeseilla* (7.36%), *Provedencia* (6.49%) and the lowest frequency was *Bacillus*

Boiled rice samples contained total nine genera and among them the prevalence of the bacteria were *Enterobacter (*23.75%) fo, 18.75 for *Citrobacter* sp., 12.5% for *Bacillus* sp., 10% for *Proteus* sp. The most three dominant bacteria were *Enterobacter*, *Citrobacter* and *Bacillus* and which contributed 55% of the total bacteria in boiled rice. Cooked dal samples contained total nine genera and the prevalence of the bacteria were 27.11% for *Citrobacter*, 19.77% for *Enterobacter* sp., 18.1% for

Salmonella sp., 9.03% for *E.coli*, 9.03 *Klebseilla* sp, *Edwarseilla* sp, 5.01% for *Proteus* sp, 3.38 % for *Staphylococcus* and 1.9% for *Bacillus* sp. The most three dominant bacteria were *Enterobacter, Citrobacter* and *Salmonella* which contributed 64.98% of the total bacteria in cooked dal.

Vegetable curry samples contained total nine genera and The prevalence of the bacteria were 30.85% for *Citrobacter*, 25.53% for *Enterobacter* sp., 8.51% for *Klebseilla* sp, 7.44% for *Staphylococcus* sp, 6.91% for *E. coli*; 6.38% for *Salmonella* sp. and *Bacillus* sp. The most three dominant bacteria were *Enterobacter* sp, *Citrobacter* sp and *E. coli* which contributed 85.75% of the total bacteria in vegetable curry. Fish curry samples contained total eight genera of the bacter*ia.* The prevalence of the bacteria were 28.71% for *Proteus* sp, 20.79% for *Enterobacte*r sp., 10.79% for *E. coli* and *Citrobacter* 8.91% for *Salmonella* sp, 8.9% for *Proveidencia* sp, 5.94% for *Edwarseilla* sp, 4.95% for *Staphylococcus* sp. The most three dominant bacteria were *Proteus* sp, *Enterobacter* sp, and *Citrobacter* sp which contributed 60.38% of the total bacteria in fish curry.Chicken curry samples contained total nine genera and The prevalence of the bacteria were 29.44% for *Citrobacter* sp, 12.26% for *Salmonella* sp., 11.65% for *Provedencia* sp, 11.1% for *E. coli,* 9.81% for *Proteus* sp., 8.58% for *Staphylococcus* sp; 7.36% for *Klebseilla and Enterobacter* sp; and 2.45% for *Edwarseilla* sp. The most three dominant bacteria were *Citrobacter* sp, *Salmonella* sp and *Proveidencia* sp which contributed 53.35% of the total bacteria in chicken curry . Fuchka samples contained total six genera and The prevalence of the bacteria were 33.3% *for Staphylococcus* sp, 23.08% for *Enterobacte*r sp; 14.28% *E. coli* and *Citrobacte*r sp; and 11.1 % for *Salmonella* sp. and 3.17% for *Klebseilla* sp. The most three dominant bacteria were *Enteobacter* sp , *Staphylococus*

sp *and Citrobacter* sp which contributed 71.38% of the total bacteria in Ffuchka. Chatpoti samples contained total five genera of the bacter*ia and* The prevalence of the bacteria were 38.7% for *Enteobacter* sp, 33.87% for C*itroboracte*r sp, 11.3% for Staphylococcus sp, 9.67% for *E. coli*, 6.45% f*Klebseilla* sp. The most three dominant bacteria were *Citrobacter* sp, *Enteobacter* sp and *Staphyloccus* which contributed 83.87% of the total nt

In overall bacterial distribution, it was observed that *Citrobacter* sp was the most frequent genera of RTE food samples sold in Rajshahi Metropolis and showed 23.3% of total abundance. Next of most dominating bacteria were *Enterobacte*r (22.19%), *Salmonella* sp. (9.47%), *Proteus* sp. (8.7%), *Klebseilla* sp. (5.78%), *Edwarseilla* sp. (3.68%), for *Proveidencia* sp(3.24%). The least dominating bacteria was *Bacillus* sp (3.07%). On the other hand, only four genera i.e. *Enterobacte*r sp, *Citrobacter* sp, *E. coli* and *Staphylococcus* sp were the most prevalence bacteria found in all types of RTE food items. *Salmonella* sp was absent in chatpoti, *Proteus* sp was absent in fuchka and chatpoti; *Klebsilla* sp. was absent in fish curry; *Proveidencia* sp. was absent in boiled rice, cooked dal, vegetable curry, fuchka and chatpoti; and *Bacillus* sp. absent in fish curry, chicken curry, fuchka and chatpoti.

Pathogenecity test of the selected isolates of different RTE foods

The pathogenicity test was carried out by hemolytic activity test on blood agar medium, direct ingestion in albino mice and hemagglutination test. Out of 62 isolates, nine isolates i.e. SMRS-651 (*Proteus vulgaris*), RKFE-227 (*Proveidencia*), SMRS-671 (*Salmonella*), BAFM-417 (*Enterobacter*), RSVE-185 (*E. coli)*, BHVM-455 (*Citrobacter*), CMCN-243 (*Enterobacter)*, RKRM-211 (*Enterobacter)* and LTDS-79 (*citrobacter)*

showed positive β-hemolytic activity. These Nine β-hemolytic positive isolates were considered for virulence test on mice. Among the isolates, SMRS-671 (*Salmonella)*, BAFM-417 *(Enterobacter)* and RSVE-185 (*E. coli)* no isolates showed highly toxic reactivity and the mice were died within 3 days and as well as SMRM-651 (*Proteus vulgaris)* and RKFE-227 (*Proveidencia)* no. isolates ingested mice also died within 6 days and rest of the isolates showed severe to mild illness symptom. All the isolates also showed positive results for hemagglutination.

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