**ANTIBIOGRAM OF BACTERIAL ISOLATES FROM COMMON STREET FOODS SOLD IN DHARAN, SUNSARI, NEPAL**



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**Department of Microbiology, Central Campus of Technology,** Tribhuvan University, Dharan, Nepal

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 **(Environment and Public Health)**

By:

**SUMIRA GAUTAM**

Roll No: 25659

TU Registration No: 5-2-8-82-2009

Central Campus of Technology

Tribhuvan University, Dharan, Nepal

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RECOMMENDATION

This is to certify that **Mrs. Sumira Gautam** has completed this dissertation work entitled **“ANTIBIOGRAM OF BACTERIAL ISOLATES FROM COMMON STREET FOODS SOLD IN DHARAN, SUNSARI, NEPAL”** as a partial fulfillment of the requirements of M.Sc. degree in Microbiology (Environment and Public Health) under our supervision. To our knowledge this work has not been submitted for any other degree.

**…………………………….**

Mr. Hemanta Khanal

Supervisor / Asst. Prof.

Department of Microbiology,

Central Campus of Technology

Tribhuvan University,

Dharan

# CERTIFICATE OF APPROVAL

On the recommendation of the supervisors Mr. Hemanta Khanal this thesis work of **Mrs**. **Sumira Gautam,** entitled **“ANTIBIOGRAM OF BACTERIAL ISOLATES FROM COMMON STREET FOODS SOLD IN DHARAN, SUNSARI, NEPAL”** has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirements for M.Sc. degree in Microbiology **(Environment and Public Health)**.

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| --- |
| …………………………….Mr. Hemanta KhanalAsst. ProfessorM. Sc. Microbiology Program CoordinatorDepartment of MicrobiologyCentral Campus of TechnologyTribhuvan University, Dharan |

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…..……………

Sumira Gautam

Date: ………………..

# ABSTRACT

Street foods are ready to eat food prepared and sold by the vendors and the hawkers in the street and public places. Pathogenic bacteria are the most commonly known causes of food contamination and food borne illness. This study therefore aimed to detect the prevalence of food borne bacteriological pathogen of ready-to-eat foods sold on various parts of Dharan Sub metropolitan city. Street food sector in Nepal operates in an unstable and precarious state as it lacks legal recognition. There have been noticeable increases of food vendors in Dharan municipality, who sell both raw and cooked food items. They operate haphazardly without any monitoring of what they prepare and how they do it. Microbial contamination of ready-to-eat foods and beverages sold by street vendors and hawkers has become a global health problem. A study to assess the microbiological status of such street foods was carried out. 60 different samples of each four various street food were collected, and microbiological analysis was carried out to enumerate total plate count, total coliform count and *Salmonella*. The average TPC were found to be 76x107, 48x107, 31x107and ,21x107, cfu/g in *aalunimki, panipuri*, *chatpate* and *dahi bada* samples respectively. Similarly, the average coliform counts were 36x107, 29x107, 21x107, and 19 x107, cfu/g in aalunimki, *panipuri*, *chatpate* and *dahibada* samples respectively. *Salmonella* was detected in 22 food samples. All the isolated bacteria pathogens were subjected to antibiotic susceptibility test to the 7 different antibiotics namely Amoxycillin, Amikacin, Cefoxitine, Chloramphenicol, Nalidixic acid, Tetracycline and Ciprofloxacin. All the isolates showed resistance to at least two or three antimicrobial and resistance to a wide range of antibiotics was observed. Strict hygienic practice, such as education of food handlers in improving the hygienic practice, regular monitoring and supervision by local authorities, sanitation and awareness health education etc. are needed in order to avoid any food-borne pathogenic outbreaks in future.

**Key words:** Street food, Pathogens, Hygiene, Microbiological Analysis

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# ABBREVIATIONS

CBS :Central Bureau of Statistics

CCT :Central Campus of Technology

cfu/g :Colony forming units per gram

CNN :Cable News Network

*et al.* :and others

FAO :Food and Agricultural Organization

HACCP :Hazard Analysis and Critical Control Point

hrs :Hours

CLSI :Clinical Laboratory Standards Institute

DM :Dharan Municipality

ng :Nanogram

p.m. :Post Meridian

EMBA :Eosin methylene Blue Agar

TCBS :Thiosulfate citrate Bile Salts-sucrose Agar

TPC :Total Plate Count

SSA :Salmonella-shigella Agar

WHO :World Health Organization

# CHAPTER I

# INTRODUCTION

## 1.1 Background

Street foods are ready-to-eat foods and beverages prepared and or sold by vendors or hawkers especially in the streets and other similar places by street food venders and hawkers.

 At present, large numbers of urban populations are relied on street food throughout the world. Street foods may be the least expensive and most accessible means of obtaining a nutritionally balanced meal outside the home for many low-income people, provided that the consumer is informed and able to choose the proper combination of foods. These days, Street foods have become increasingly popular all over the world and Nepal is no exception to it. The increasing demands of the street foods are due to its taste, flavor as well as its varieties. Mostly in developing countries, street food preparation and selling provides a regular source of income for millions of people with limited education, skills and experiences.

Dharan municipality (19,261 sq.km area) is a sub-metropolitan city in Sunsari district of Province no.1 of eastern Nepal. It is situated at the foothills of the Mahabharat range in the north with its southern part touching the terai region. It is the connecting city of northern hilly region and thus is a town of emerging importance. The population is increasing rapidly resulting in an increasing problem with regard to sanitation, hygiene, availability of clean water and food hygiene (DM, 2008). Street foods are sold all over the city, especially concentrated in the market area.

“Eating out” has become an inseparable fact of city life today- driven by the imperatives of urban lifestyles, occupational demands and consumer convenience. Maintenance of appropriate health and hygienic standards is becoming a valid concern. Although many consumers attach importance to hygiene in selecting a street food vendor, consumers are often unaware of the health hazards associated with street vended foods.

Street foods in these days are an important source of affordable and ready to eat food available to everyone. Street food plays an important socioeconomic role in meeting food and nutritional requirements of city consumers at affordable prices to the lower and middle income groups and convenience.

Street food also provides food security for low income urban population in many developing countries. In the past, people have been consuming only home-made foods, but nowadays people have been attracted towards the hotels, restaurants and street foods. Street foods are described as wide range of ready to eat foods and beverages which can be consumed without further preparation. As the urban population of Nepal is increasing rapidly, the demand of street food is also increasing highly. The low cost, accessibility, and convenience are the key factors for growing popularity of street foods. Street food ingredients are area specific and mostly undocumented. There are different varieties of street food that it is impossible to provide menu of all the street food consumed around the world. These foods are usually sold by vendors and hawkers in the streets or other public places. Street vended foods are not only appreciated for their unique flavors and convenience but they also important in contributing to the nutritional status of the population. In contrast, to these potential benefits, it is also recognized that street food vendors are often poor, uneducated and lack of knowledge in safe food handling environment, sanitation and hygiene, mode of food display, food service and hand washing, sources of raw materials and use of potable water. Street –vended foods are predisposed to contaminations because they are sold in the open places and often not covered. Likewise, since street vendors take their products to their customers, they often operate from busy places such as bus parks, industrial areas, schools, market places and streets. These places often do not meet food and safety requirements. Sale of food in the open spaces without meeting basic safety requirements is very risky from health point of view. As, a result street food are perceived to be a major public health risk.

 The food habit among the people at Dharan has vast demarcation due to the various groups of people and also their busy life. Some common street foods that are sold in Dharan municipality are *Chatpate*, Aalunimki ,*Dahibada*, Panipuri, *Aluchop*, etc. Street foods are mainly found where majority of flow of people occurs such as Bus Park, chowk, school area, main market and street. Almost most of the people are attracted towards street food but population of age group 10-35 years are found to be fond of it. These street foods undergo contamination by microorganisms as well as sand, dust, clay, unsanitized utensils and the use of water of unsatisfactory quality, due to which serious problems occur sometime. The quality of the raw materials used also has significant influence of the quality of street food. Some of the commonly occurring diseases due to contamination are diarrhea, dysentery, cholera, fever, etc. Consumers are also responsible for this deteriorating condition. The risk of serious food poisoning outbreaks linked to street foods remains a threat in many parts of the world. A lack of knowledge among street food vendors about the causes of food borne disease is a major risk factor.

The quality of food needs to be evaluated before consumption. Basically, street foods are not sold under hygienic condition. They are freely in contact with the environment and other agents and no preventive measures are taken. The microbiological quality of food is important from public health point of view. Both the street food venders and consumers of Dharan are not very sensitive towards the food quality. Mostly in developing countries, food poisoning incidences due to consumption of poor-quality food has not been recorded to date, this does not necessarily imply that the street foods sold in Dharan is safe. Due to this, an investigation is necessary for the safety of street foods.

## 1.2 Objectives

### 1.2.1 General Objectives

To study the Antibiogram typing of bacteria isolates from common street food sold in Dharan Sub- Metropolitan city, Sunsari

### 1.3.2 Specific Objectives

1. To determine the extent of microbial contamination in common street foods sold in Dharan, Sunsari, Nepal.
2. To isolate and identify the pathogenic flora of street foods, e.g. *Salmonella*, coliforms, etc.
3. To evaluate the antibiotic susceptibility pattern of isolated bacterial pathogens.

## 1.4 Importance of Study

In order to manage and control the microbiological hazards associated with the Street food, consequently FAO of the United Nations has pointed out the needs for proper sanitation and hygiene of Street food. Since Street food enterprise has been neglected in research, even though it takes up a part of food service industry. There are lots of efforts need to be made to ensure food sanitation and safety. Street food plays an important role in developing countries in meeting the food demands of the urban population. It is estimated that street foods contribute up to 40%of the daily diet of the urban consumers in developing countries like Nepal. Street foods are consumed by thousands of populations daily with the wide variety of foods that are relatively cheap and easily accessible. Most of the street food vendors are with little education and training. Thus, poor personal and environmental hygiene contribute significantly to food contamination and resultant food borne illness. Since, this study aims to isolate the common food borne pathogens of Street food of Dharan. The present work will generate adequate data giving true picture about the microbial contamination of street foods vended in Dharan.

Upon analysis of the data, the work will be able to recommend techniques and ideas for Dharan Sub-Metropolitan city for providing safe Street food as well as public awareness campaign at local level. The outcome will be fruitful to all those who are directly or indirectly concerned with food and health risk sector.

## 1.5 Limitations

a. Identification and confirmation of street food borne bacterial pathogens by molecular method could not be done because of cost problem.

b. Due to limited laboratory facilities Serotyping of isolates could not be performed.

c. Parasitic pathogens,viruses and bacterial toxins were not investigated in this study.

d. The study was limited to the investigation of some common pathogenic bacteria like *Staphylococcus* spp. *Salmonella*, *E. coli*,*Vibrio* species and indicators like coliform only.

**CHAPTER II**

# LITERATURE REVIEW

## 2.1 Street Foods

Street foods are defined as “foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation (WHO, 1996).

Street food is ready-to-eat foods or beverages, which includes many types of foods ranging from cereal and fruits to cooked meats and drinks. It is usually sold in public premises, such as school area, busy street, markets, cinema hall premises, bus and railway stations, beaches, parks and other public spaces. Street foods are served with the minimum individual portions dished into take-away containers like paper, disposable plates and utensils. Normally these containers come in a variety of materials such as disposable plastic, paper, bowls, cups and utensils (Anon, 2008).

### 2.1.1 Some Common Street Foods

 The popularity of street food is increasing all over the world and Nepal is no exception to it. Street foods represent a significant part of urban food consumption for millions of low-and-middle-income consumers, in urban areas on daily basis. As concerned to Dharan municipality, some commonly sold street foods are:

1. ***Chatpate:*** It is a product which contains the mixture of raw vegetables, puffed rice, onions, tomato, salt, chili powder, oil, lemon juice additives color and other spices. All the ingredients are placed together in a vessel and mixed properly. The varying quantities of salt, lemon juice and spices cater to the taste (PC,2010)
2. ***Panipuri*:** It is a concoction of crispy fried semolina falls filled with seasoned mashed potatoes and lentils. It is also known as *golgappa*, *puchka* and *gupchup*. It is a popular street snack which comprises a round, hollow, fried crisp and filled with a mixture of water, tamarind, chili, *chaat masala*, potato, onion and chickpeas. The word p*ani* comes from the Nepali/Hindi word for water and *puri* is the bread made by frying dough in oil (Wikipedia, 2010b)
3. ***Aalunimki:***It is a dish made with potato curry and flour crackers (Nimki) popular in eastern part of Nepal especially Dharan. The potato is cut into small pieces after boiling and peeling off. The flour cracker (Nimki) is mixed with it adding various other ingredients like salt, pepper, chilies and peas.
4. ***Dahibada*:** It is a popular South Indian dish. It consists of dahi (yoghurt) and *bada.* For *bada*, clean*daal* is taken, washed and soaked overnight and then grinded into smooth paste. Salt is mixed accordingly. Oil is heated in a pan and fried till golden brown. Hot *badas* are put in cold water for 2-3 minutes and then water is squeezed. Dahiis blended with water until it is smooth. Salt, red chili powder and cumin powder are added. Finely chopped coriander leaves and green chilies may also be added. It may be chilled by keeping in refrigerator as well. After the preparation of both parts, *badas* are arranged in deep dish and dahi poured over them (Anon, 2010b).

### 2.1.2 Role of Street Foods in Diet

Street foods have significant nutritional implications for consumers, particularly low- & middle-income sectors of the population who depend heavily on street foods. Therefore, street food alleviates food insecurity from these classes of people to large extent. So, in this respect, street food vendors can be called as the nutritionist of the poor. However, a number of factors influence the consumer’s choice which plays an important role. These include cost, convenience and type of food available, the individual’s taste and the organoleptic qualities of food (smell, texture, color and appearance). The nutritional value of street food depends upon the ingredients used and how they are prepared, stored and sold (Chakravarty and Chittranjan, 1995).

A study conducted in India showed 1000 calorie meal contained about 30 gm protein, 15 gm fat and 180 gm carbohydrate. It is estimated that the recommended daily energy intake can be met by consuming street foods which cost approximately US$ 1. Several foods such as boiled and fried peanuts, fried tempeh, and fried tofu are good source of proteins and fats are foods of animal origin such as chicken barbecue, fried fish and other local meat, fish dishes. If such foods are complemented by others, one can testify to the good nutritional value and quality of street foods (Chakravarty and Chittranjan, 1995).

### 2.1.3 Status of Street Foods in Asia

Large number of populations is involved in street food industry directly or indirectly. It is estimated that 100,000 street food vendors are employed in Malaysia and a million in China. In Indonesia, 26% of the informal sector is directly or indirectly involved in street food industry. In Malaysia and Philippines, 25-30% of the household expenditure is spent on street foods (FAO, 2001a).

According to Sharma (2007), Nutrition Foundation of India (NFI) conducted a study in late nineties on safety and nutritional implications of the consumption of street and convenience foods in urban areas. NFI gathered information of the street food industry, the socio-economic status of street food sellers and consumers and various aspects such as safety, mode of preparation, handling practices, storage, and distribution of street foods. The study revealed the following interesting features of the street foods industry:

- Vendors between the age of 18 and 40 years actively participate in all aspects of the street foods industry, such as purchase, preparation and distribution.

* Almost 43% of the street food vendors were illiterate and majority of them were not familiar with nutritive value of foods and they were not familiar with modern methods of food processing, handling and how to ensure food safety.
* 44% of the vending points were not properly cleaned by the vendors and ash, coal particles, dust, garbage etc. were reported there.

Rapid change in urban lifestyles, occupational demands and convenience, home cooked meals being replaced with more 'eating out' in canteens, cafeterias and restaurants, food joints, fast food corners and in a large number of street vending stalls. Threats to consumer health include lack of clean water, contamination of foodstuffs by dust and airborne pollutants, poor hygiene, improper storage, deteriorating urban environments and, finally, the threat of communicable diseases being spread via the food system. The risks of food contamination are even more evident in hot and humid climatic zones like ours, which make ideal breeding grounds for water and food-borne bacteria.

## 2.2 Safety Aspects of Street Foods

Street foods are consumed by an estimated two and a half billion people world-wide. Because of its low cost and convenience, street food is an indispensable part of urban and rural diets in the developing world. But there are also risks. Food stalls often lack the necessary storage, refrigeration and cooking facilities to prevent contamination with bacteria such as *Salmonella*. In warm, moist conditions, a single bacterium can duplicate into 17 million disease-bearing organisms in just eight hours. And limited access to running water and waste disposal increases the potential for passing the problem to many customers (FAO, 2001b).

FAO studies on street food have highlighted a number of food safety problems and issues due to the lack of education and little or no knowledge of good hygiene practice in the handling and preparation of food among vendors. Furthermore, they work under crude and often unsanitary conditions; therefore the contamination of foods is almost bound to happen. The potential hazards of street vended foods may be the possibility of microbiological, chemical and physical contamination, which conceivably could occur under street conditions. Among these three different types of hazards, the most threatening hazard would be the microbiological hazards. The others are environmental contaminants, naturally and occurring toxicants, pesticide residues, and food additives which can be present in street foods (FAO/WHO, 2003).

### 2.2.1 Microbiological Safety

Microbiological contamination is the major problem associated with street foods. According to the nature of street food and condition in which it is held and the manner in which it is served, the associated risk may vary considerably. Studies have demonstrated the presence of unacceptably high level of microbiological contamination and the presence of pathogenic bacteria such as *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens* or *Vibrio cholerae*. Contaminated water has frequently been shown as an important source of contamination in street foods (Codjia, 2000).

Mensah*et al*. (2002) reported that of 511 street food items examined in Accra, 69.7% contained mesophilic bacteria, 5.5% contained *Bacillus cereus*, 31.9% contained *S. aureus* and 33.7% contained Enterobacteriaceae. *Shigellasonnei* was isolated from macaroni, *Salmonella arizonae* from meat-based soup and *E. coli* from macaroni, tomato stew and rice (Mensah*et al*., 2001). In a separate study, it was observed that over 26% of street food samples analyzed in Nigeria contained *B. cereus*, while 16% contained *S. aureus* (Umoh and Odoba, 1999). These observations indicate that although street foods are a major source of nutritious food, they are also a possible source of food poisoning microorganisms. For example, 14 deaths reported in the Malaysian state of Perak were attributed to the consumption of rice noodles bought from different vendors. In 1981, a cholera epidemic in Pune city, India, was attributed to contaminated sugar cane juice with added ice. In this case, the ice was found to be contaminated with *Vibrio cholerae*.

### 2.2.2 Artificial Coloring Matters

Artificial colors are mostly added in a street food in order to enhance taste and increase attractiveness of food. Foods containing these dyes are not suitable for consumption because these dyes contain lead, mercury and many other hazardous chemicals which can accumulate within the human body and cause cancer. In addition, these dyes can cause hindrance in digestion and absorption of nutrients from the intestinal tract (FAO, 1988).

Most of the Vendors are not aware of the regulations pertaining to artificial colors, that lists the approved colors, the foods that can contain them and the amount that can be used (not to exceed 200 ppm). For instance, metanil yellow (a textile colour) has a long history of use as substitute for saffron. Many users of metanil yellow do not know that it is not permitted. Unauthorized use of food additives is found especially in foods such as sherbets (cold sweetened milk-based desserts), *jalabi* (extruded cereal batter fried and dipped in sugar syrup) and other sweets such as *laddu, kamalabhog* and *pantua*. In addition to containing very bright non-permitted colors, various food samples, especially certain sherbets, also contained saccharin. Saccharin is generally not permitted in foods except in a small amount of 100 ppm which can be added only to carbonated water (Chakravarty and Canet, 1996).

### 2.2.3 Personal Hygiene

Purchasing ready-to-eat foods and ingredients from street/market vendors poses a considerable risk to public health, especially due to the observed poor hygienic practices. In most cases where studies of street food vending have been done, the vendors do not have adequate washing facilities, and some vendors started their duties without taking a proper bath. Some of the vendors sleep at the vending sites in order to protect their wares. Foods and ingredients are also subjected to repeated contamination from unwashed hands and the materials used for wrapping, such as leaves, old newspapers and reusable polyethylene bags (Ehiri*et al*., 2001).

### 2.2.4Environmental Hygiene

Street food vendors usually set up their stall in overcrowded areas where there are high numbers of potential customers. Such areas usually provide limited access to basic sanitary facilities

Poor sanitary conditions in the area where foods are vended also contribute to poor food storage and transport conditions. Some of the street food vendors obtain their raw materials and other condiments from licensed shops, and therefore there is less concern regarding the safety of these raw materials. However, most of the vendors have no fixed stalls where they can store their raw materials on site. They usually store their goods at home overnight and transport them the following day, often improperly covered, to their operating sites. Thus, the food becomes prone to contamination during transportation (FAO/WHO, 2003).

## 2.3 Microbiology of Foods

In most cases, microorganisms use our food supply as a source of nutrients for their own growth. This, of course, can result in deterioration of the food. When the microorganisms involved are pathogenic, their association with our food supply is critical from a public health point of view.

**2.3.1 Source of Contamination**

The probable source of contamination is mention below,

1. Nasal and throat discharge of sick individuals or asymptomatic carriers;
2. Infections on body surfaces of food handlers (hands and arms);
3. Infected soil, mud, surface water, dust; and
4. Mixing of raw vegetables with cooked food.
5. Knifes, lack of sanitation, utensils, papers.
6. Personal and environment source.
7. Insufficient resources for inspection and laboratory analysis
8. Poor knowledge of street vendors in basic food safety measures.
9. Difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary

The negative aspects of street vended foods relate primarily to food borne hazards associated with the food as well as the obstruction of pedestrian and vehicular traffic and littering (FAO/WHO (2003). The inability of maintenance of proper hygienic conditions in and around street foods to is more prone to microbial contamination.

**2.3.2Microorganisms Associated with Food**

 Microorganisms are present from contamination of the food at some stage of production, harvesting, handling, processing, storage, distribution, or preparation for consumption. Most foods are subjected to potential sources of microorganisms, which include soil, air, water, plants, feed or fertilizer, sewage, animals, human beings, processing equipment, ingredients, product to product, and packaging materials. Microorganisms can be exchanged between these various sources. Flies also play an important role in the transmission of disease-causing organism. Microorganisms isolated from various food samples include genera of *Staphylococcus, Brevibacterium, Micrococcus, Bacillus, Lactobacillus, Corynebacterium, Flavobacterium, Pseudomonas, Alcaligenes, Streptococcus, Pediococcus, Acinetobacter, Microbacterium, Salmonella, Clostridium, Vibrio,* various coliforms and yeasts. The same genera found on red meat tend to be present in poultry, animal products such as milk and eggs, and fishery products. Many types of microorganisms are associated with plant products. The most common bacteria are genera of *Leuconostoc, Lactobacillus* and *Erwinia*. Other genera include *Aeromonas, Alcaligenes, Bacillus, Corynebacterium, Flavobacterium, Pseudomonas, Klebsiella, Serratia and Xanthomonas.* Molds on vegetables include M*ucor, Alternaria, Aspergillus, Aureobasidium, Botrytis, Chaetomium, Cladosporium, Epicoccum, Penicillium, and Rhizopus* (Banwart, 2000a).

Soil, water and air are the natural habitat of many microorganisms. Those that are common in food and found in soil include genera of *Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Clostridium, Corynebacterium, Flavobacterium, Pseudomonas, Micrococcus* and *Streptomyces.*

**2.4 Microbial Hazards in Foods**

It must be borne in mind that the presence of microorganisms in foods is not necessarily an indicator of a hazard to the consumer. Plants and animals form the major origin of the foods, which we eat, and these sources are naturally associated with a microorganism, which implies that foods will be associated with microorganisms. Food can become microbiologically hazardous to the consumer when the principles of hygiene and sanitation are not met or when it becomes contaminated by pathogens from humans or from the environment during production, processing or preparation, or when it originates from a sick animal. On subjection to conditions that allow the entry and/or growth of infectious agents, it may become a vehicle for transmission of diseases such as Salmonellosis or Staphylococcal food poisoning. Examinations of food samples allow us to determine the presence of these hazards (Anon, 2009b)

#### 2.4.1Plate Counts (total viable counts / aerobic mesophilic plate counts)

Counts of viable bacteria are commonly based on the number of colonies that develop in nutrient agar plates which have been inoculated with known amounts of diluted foods and then incubated under prescribed environmental conditions. Only those bacteria which will grow under the chosen environmental conditions are counted (expressed as colony forming unit per gram i.e. cfu/g). A wide variety of conditions can be obtained by changing the composition of the growth (agar) medium, the gaseous environment of incubation (presence or absence of oxygen) and the time and temperature of incubation. The aerobic mesophilic count is most commonly used.

#### 2.4.2 Coliforms

The presence of enteric bacteria, e.g., coliforms in general and *Escherichia coli* in particular have been widely accepted as indicators of fecal contamination (not in the sense of implying immediate contact with the feces) and therefore the indicators of the possible presence of pathogens of enteric origin, e.g., *Salmonella*.

While the presence of large numbers of coliforms and *E. coli* in foods is highly undesirable, it would virtually be impossible to eliminate all of them from fresh and frozen foods. Low numbers of coliforms are usually permitted in sensitive foods at numbers ranging from 1 to not exceeding 100/g or ml. The finding of *Escherichia coli* higher than 102cfu/g indicates dangerous contamination of food (Anon, 2009b).

The presence of considerable numbers of coliforms in processed foods indicates:

1. Inadequate processing and/or post process recontamination due to cross-contamination by raw materials, dirty equipment or poor hygienic handling;
2. Microbial proliferation, which could have allowed multiplication of a wide range of pathogenic and toxigenic organisms (Anon, 2009b).

#### 2.4.3 Yeasts and Molds

 Yeast and molds grow slowly in non-acid, moist foods, and therefore rarely cause problems in such foods where as in acidic foods and foods of low water activity, they outgrow bacteria and thus cause spoilage losses especially if the products (e.g., vegetables fruit juice and dried foods) are improperly stored. It is harmful to consume the mouldy food. Consumers will recognize spoilage when very high numbers of yeast or visible moulds are present (Anon, 2009b).

### 2.4.4 Foodborne Pathogens

There are numerous cause of food borne diseases e.g. nutritional deficiencies; over eating, poisoning by chemicals; toxins produced by bacteria; infestation by animal parasites; and infection by microorganisms .Food poisoning is consider as the diseases caused by microorganisms, which include illness caused by the ingestion of toxins elaborated by the organisms and those resulting from infection of the host through the intestinal tract. All food borne diseases are subdivided into poisonings and infections. *Salmonella, Campylobacter jejuni, E. coli, Shigella* spp.*, Yersinia enterocolitica, Vibrio parahaemolyticus, Vibrio cholera, Aeromonas, Clostridium perfringens* and *Listeria monocytogens* are responsible for food borne infections. *Staphylococcus aureus, Bacillus* spp., *Clostridium botulinum* and other protozoa and viruses are responsible for food borne intoxications. The majority of outbreaks and causes are attributed to Staphylococcal intoxication, Salmonellosis and *Clostridium perfringens* gastroenteritis (Fraizer and Westhoff, 2008e).

#### 2.4.4.1 *Salmonella*

*Salmonella* are potentially pathogenic for humans as well as for other animals. Direct transmission is possible from human to human, from human to animal and from animal to human (Banwart, 2000c). The transmission of the disease is usually from animals to humans by the ingestion of food of animal origin

*Salmonella* occurs worldwide and it is recognized as a zoonotic agent. The primary habitat is the intestinal tract of animals including humans. Ingestion of certain strains of *Salmonella* can result in foodborne disease. Foods that are commonly identified as vehicles of salmonellosis to humans include eggs, poultry, meat and meat products. The food-poisoning syndrome is generally due to the ingestion of foods that contain significant numbers of certain serotypes of *Salmonella*. Normally levels necessary to cause salmonellosis range from 107-109 cells/g. Levels of 105/g is highly suggestive of the possibility of food poisoning occurring (Anon, 2009b).

#### 2.4.4.2 *Clostridium perfringens*

*C. perfringens* has been called ubiquitous, due to its widespread distribution in nature. It is found in soil, dust, air, water, sewage, human and animal feces, and on many food products. Good growth occurs between pH 5.5 and 8.0. The optimum range for enterotoxin production is pH 6.5 to 7.3. *C. perfringens* grows rapidly at temperatures between 20o and 50oC, with maximum growth between 37o and 47oC. These organisms produce spores that are relatively heat stable which influences their survival during and after cooking. Those that survive will grow and multiply especially during poor storage conditions and cause food poisoning. Food poisoning caused by this organism is relatively mild.

#### 2.4.4.3 *Escherichia coli*

*E. coli* is an important organism in the microbiology of foods. This organism is considered as reservoir of intestinal tract of human, cattle and other animals. However, fecal contamination causes it to spread to other environments, especially soil and water. It is widely distributed in food environments in low numbers. Low doses cause illness in young children, the elderly and immune-compromised persons. Foods implicated include undercooked hamburger patties and other fast foods and cheese made from unpasteurized milk (Anon, 2009b).

#### 2.4.4.4 *Listeria monocytogenes*

The organism is a small gram-positive rod with a tendency towards a diplobacilli form. Although, it is aerobic, it grows better at reduced O2 and increased CO2 levels. It is ubiquitous in nature (i.e. widespread in soil, food-processing environments, raw meats and feces of healthy humans and animals). It is an opportunistic pathogen affecting mainly the elderly, immuno-compromised persons, pregnant women and young children. The minimal infectious dose is estimated to be >102/g. It has the ability to grow even at refrigeration temperatures, which makes it a problem in refrigerated foods. Foods normally implicated in outbreaks include soft cheeses, pates, fermented sausages and coleslaw and other salads (Anon, 2009b).

#### 2.4.4.5 *Shigella* spp.

*Shigella* spp. is not the natural inhabitants of the environment. Normally, they are found in intestinal tract of human beings and other primates. They are rarely found in other animals. These are considered as host adapted organisms and only infect humans and other primates. Foods such as milk, vegetable salads, juice and cooked rice which serve as vehicles. The infective dose is small (101 - 102/g). The main source of *Shigella* involved in outbreaks is people who are symptomless carriers, or ambulant cases. Foodborne outbreaks of shigellosis are caused by the mishandling of food (Anon, 2009b).

#### 2.4.4.6 *Vibrio* spp.

Three species of importance are *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. The first two species cause gastroenteritis while the latest causes diarrhoea in humans. These are widely distributed in rivers and marine environments. *V. cholerae* 01 counts of >107/g normally cause disease in healthy persons. *V. parahaemolyticus* and *V. cholerae* are typically present in sea foods at levels below 103/g. Foods implicated in outbreaks are usually raw oysters and other molluscan shellfish (Anon, 2009b).

#### 2.4.4.7 *Staphylococcus aureus*

*S. aureus* is found in the mucous membranes and skin of most warm-blooded animals, including humans. Unless heat processing steps have been applied, this opportunistic pathogen may be expected to exist in low numbers in many food products that are of animal origin or in those that are handled directly by humans. It does not compete well with other bacteria. It is seldom linked to food poisoning outbreaks from consumption of raw products. It can be readily killed by cooking, but toxins are heat stable and will survive. It is resistant to freezing and thawing, and survives well in foods stored at –20oC, but at higher temperatures ranging from –10oC to 0oC the viability of these cells decreases markedly during frozen storage. The minimum number of cells of *S. aureus* required to produce the minimum level of enterotoxin considered necessary to cause the gastroenteritis syndrome in humans depends on the substrates. The minimum quantity of enterotoxin needed to cause illness in humans is about 200 ng. Counts of 105/g are highly suggestive of the possibility of food poisoning occurring (Anon, 2009b).

#### 2.4.4.8 *Bacillus cereus* and other *Bacillus* spp.

*B. cereus* is an aerobic, gram-positive, motile, spore forming rod. It is widely distributed in nature. It is common in soil and dust, so it is logical that foods that are readily contaminated by soil and dust will also contain the organism. Plant products (cereals, flours, starch, bakery products, and spices), animal products, and mixtures of ingredients (spaghetti, sauce, pudding, soup mixes, gravy mixes) can contain a few or many cells or spores of *B. cereus*. It can survive all food processing, except retorting (canning). The organism is present in most raw materials used in food manufacture. It is normally found in food in concentrations of 103/g or less, but mostly at levels less than 102/g. Under normal circumstances (i.e. at these concentrations), *B. cereus* is not considered to be innocuous. The infectious dose has been estimated to be >105/g (Banwart, 2000c).

#### 2.4.4.9 *Clostridium botulinum*

It is widespread in nature and spores are widely distributed in soil. This rod-shaped soil bacterium is saprophytic, spore forming, gas forming and anaerobic. Seven types are distinguished on the basis of the serological specificity of their toxins (viz. Type A-G); the predominant (or only) toxin from these types is designated by the same capital letter. It produces a heat-labile toxin, which is considered to be the most toxic of all naturally occurring substances. Levels of between 0.1 and 1.0 ng of toxin A have been estimated to cause death.

## 2.5 Key findings of the WHO survey of street vended foods

In 1993, the World Health Organization through its six Regional Offices undertook a survey of its Member States to assess the current situation in regard to street-vended food and to obtain the views of responsible authorities concerning the hazards posed by street-vended foods and contributing factors, as well as approaches for managing these hazards. Over 100 countries participated in this survey which represents the most extensive report on street-vended food available to date. The survey noted that almost all countries reported a wide variety of foods, types of preparation, facilities and infrastructure (WHO, 1996).

1. 74% of countries reported street-vended foods to be a significant part of the urban food supply;
2. Street-vended foods included foods as diverse as meat, fish, fruits, vegetables, grains, cereals, frozen produce and beverages;
3. Types of preparation included foods without any preparation (65%), ready-to-eat food (97%) and food cooked on site (82%);
4. Vending facilities varied from mobile carts to fixed stalls and food centers;
5. Infrastructure developments were relatively limited with restricted access to potable water (47%), toilets (15%), refrigeration (43%) and washing and waste disposal facilities;
6. The majority of countries reported contamination of food (from raw food, infected handlers and inadequately cleaned equipment) and time and temperature abuse to be the major factors contributing to food borne disease; and
7. Most countries reported insufficient inspection personnel, insufficient application of the HACCP concept and noted that registration, training and medical examinations were not amongst selected management strategies.

**CHAPTER III**

# MATERIALS AND METHODS

The study was carried out in the microbiology laboratory of the Central Campus of Technology, Hattisar, Dharan covering the period from May to August 2017. All the laboratory works were performed in microbiological laboratory of Central Campus of Technology, Dharan, Nepal according to standard methodology.

## 3.1 Materials

The materials used in this study are mentioned in the appendix.

**3.2 Methods**

**3.2.1 Study duration**

The study was conducted from May to august 2017.

**3.2.2.1 Laboratory Set up**

All the laboratory work was done in the laboratory of Central campus of technology,Dharan.

## 3.3Area of study

The research was carried out at Dharan from May to August 2017. The study area was located in Sunsari district in the Koshi zone of south-eastern Nepal, where the climate was medium hot and rainy, and the mean daily temperature was 28°C with a range of 18-37°C. The relative humidity was as high as 98% in the mornings of wet seasons and as low as 20% in the afternoon of the dry seasons. The population of food vendors was unknown, but sale of ready-to-eat food appeared to be an important occupation of people of low economic level. The study was concentrated in the market area having poor hygienic environments which could pose serious health risks to ready-to-eat or street foods.

## 3.3 Sampling Method and sampling size

Simple random method sampling was done for the collection of sample and total 60 most common food samples were selected viz 15 aalunimki, 15 Panipuri, 15 Chatpate and 15 dahibada from different sites (Singhadevi Chowk, MachhaVaudi, Fushree, Ratna Road, Gita Mandir Line, Bhanuchowk, Bargacchi, Bijaypur, Railway and Zero point) in Dharan

**3.3.1 Sample Collection and Transportation**

A sample of 250 gram from each place was collected in sterile plastic bag and transported to lab immediately maintaining cold chain. Generally, samples were collected at 11am-1pm. The transported samples were either processed immediately else were preserved at 4°C.

## 3.4 Processing of Food Sample

### 3.4.1 Homogenization

25 grams of each sample were aseptically transferred into mortar and 225 ml sterile distilled water was also added in the same and homogeneous mixture of sample was obtained by grinding with the help of pestle. Before starting the process, mortar and pestle were thoroughly washed with clean water, distilled water and finally with 70% alcohol (Adhikari et.al 2012).

### 3.4.2 Serial dilution of homogenate

1 ml of that sample homogenate (10-1 dilution) was pipette and mixed in test-tube containing 9 ml distilled water. This was then shaken well and labeled as 10-2. From this dilution, 1 ml sample was transferred to another tube containing 9 ml distilled water and shaken well and that tube was labeled 10-3. Similarly, for 3rd, 4th, 5th and 6th tubes, the same process was repeated and the tubes were labeled 10-4, 10-5, 10-6 and 10-7respectively (KC and Rai, 2007).

## 3.5Laboratory Analysis of Sample

## 3.5.1 Total Plate Count (TPC)

Total plate count was determined by spread plate technique using plate Count agar (Himedia, India).0.1 ml of homogenate sample was taken on PCA and inoculated with the help of sterile dolly rod.

**3.5.2 *Staphylococcus aureus***

*S. aureus* was isolated by spread plate method on Mannitol salt Agar (MSA) after enrichment with normal saline and identified by biochemical tests according to Bergey’s Manual of Determinative Bacteriology.

**.3.5.3 *E. coli***

*E. coli* was isolated by spread plate method on EMBA medium (Himedia, India), after enrichment with normal saline and identified by biochemical test according to Bergey’s manual of Determinative bacteriology.

**3.5.4 *Vibrio* spp**

Vibrio was isolated by Spread plate method on TCBS medium (Himedia,India) after enrichment with alkaline peptone and identified by biochemical test according to Bergey’s manual of Determinative bacteriology.

### 3.5.5 Total coliforms count

Total coliform counts of food were determined by spread plate method using Violet Red Bile Agar (VRBA) media (Himedia, India). 0.1 ml of homogenate sample was taken on VRBA and inoculated with the help of sterile dolly rod.

### 3.5.6 *Salmonella and Shigella*

*Salmonella* was detected by enrichment of food sample with Selenite Cysteine broth then spread plate technique on SSA and identified by biochemical tests according to Bergey’s manual of bacteriology.

**3.6Antibiotic Susceptibility Test of Isolates**

AST of isolated pathogens was done by modified Kirby Bauer Disk diffusion method following CLSI guidelines (2011). For this fresh colonies were selected and transferred into NB to obtain turbidity of 0.5 Mc farland barium sulfate standard (1.5 \* 10^8 CFU/ml). MHA were inoculated and antibiotic discs were placed with sterile forceps and incubated at 37°C for16-18 hours. The zones of inhibition were interpreted as susceptible, intermediate and resistant according to CLSI “Diffusion Supplemental Table” (2013). A wide range of antibiotics were used for AST.

**3.7 Statistical Analysis**

Chi-square test for source verses *S.aureus*, source verses *B.cereus,* source verses *Salmonella*, source verses *Shigell*a, sources verses *Vibrio* was calculated with the help of SPSS (Statistical Package for Social Sciences ) version23.

###### Fig. 3.2 Flow chart for isolation and identification of *Salmonella* and *Shigella*

Sample Collection

Stored at 4°C in ice box and transported to laboratory

Homogenization 25gm of sample with motor and pestle

Enrichment with 225ml Selenite cysteine broth (37oC for 18hrs)

Spread plate on SSA(37°C for 24 Hours)

Cultural Characteristics

Gram’s Staining and microscopic examination

Biochemical Test

Identification of pathogens

Antibiotic Susceptibility Test (Kirby Disc Diffusion Method)

###### Fig. 3.3Flow chart of Isolation and Identification of *Vibrio spp*

Sample Collection

Stored at 4°C in ice box and transported to laboratory

Homogenization 25gm of sample with motor and pestle

Enrichment with 225 ml alkaline peptone water (37oC for 24hrs)

Spread plate on TCBS Agar (37°C for 24 Hours)

Cultural Characteristics

Gram’s Staining and microscopic examination

Biochemical Test

Identification of pathogens

Antibiotic Susceptibility Test (Kirby Disc Diffusion Method)

###### Fig. 3.4 Flow chart of Isolation and Identification of *Staphylococcus aureus*

Sample Collection

Stored at 4°C in ice box and transported to laboratory

Homogenization 25gm of sample with motor and pestle

Enrichment with 225 ml alkaline peptone water (37oC for 24hrs)

Spread plate on MSA (37°C for 24 Hours)

Cultural Characteristics

Gram’s Staining and microscopic examination

Biochemical Test

Identification of pathogens

Antibiotic Susceptibility Test (Kirby Disc Diffusion Method)

###### Fig. 3.5: Flow chart of Isolation and Identification of *E. coli*

Sample Collection

Stored at 4°C in ice box and transported to laboratory

Homogenization 25gm of sample with motor and pestle

Enrichment with 225 ml of normal saline (37oC for 24hrs)

Spread plate on EMBA (37°C for 24 Hours)

Cultural Characteristics

Gram’s Staining and microscopic examination

Biochemical Test

Identification of pathogens

Antibiotic Susceptibility Test (Kirby Disc Diffusion Method)

###### Fig. 3.6 Procedure of Antibiotic Susceptibility Test

Selection of fresh colonies

Transfer to Nutrient Broth equivalent to turbidity of 0.5 McFarland Barium Sulfate and mixed well

 Inoculated on MHA plates with sterile cotton swabs

Zone of inhibition were measured in mm scale

Results were interpreted according to CLSI Diffusion Supplemental Table 2013

# CHAPTER IV

# RESULTS

## 4.1 Microbiological Analysis of Street Food sample

Different 60 street food samples (15 Chatpatte, 15 Dahibada, 15 Panipuri, and 15 Aalunimki) from different locations of Dharan were analyzed microbiologically.

### 4.1.1 Total Plate Count (TPC or TVBC)

The Total Plate Count obtained for all 60 samples are listed in Table 1. The TPC were TMTC in 9 Aalunimki samples 2, 3, 4, 6, 7, 8, 9, 10 and 11. The highest TPC was found in Aalunimki sample 13 (83x107cfu/gm) and the lowest TPC was found in Chatpatte sample 8 (14x106 cfu/gm). The highest loads of bacterial pathogens were found in Machhavaudi area while the lowest bacterial load was found in zero-point area (Table 1).

Table 1: Total Viable Count of Food samples

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sample Code | TPC(cfu/gm) | Sample Code | TPC(cfu/gm) | Sample Code | TPC(cfu/gm) | Sample Code | TPC(cfu/gm) |
| A1 | 81x106 | P1 | 31x107 | C1 | 24x106 | D1 | 25x106 |
| A2 | TMTC | P2 | 75x107 | C2 | 26x106 | D2 | 32x106 |
| A3 | TMTC | P3 | 26x107 | C3 | 29x106 | D3 | 30x106 |
| A4 | TMTC | P4 | 29x107 | C4 | 30x106 | D4 | 19x106 |
| A5 | 70x107 | P5 | 29x107 | C5 | 25x106 | D5 | 22x106 |
| A6 | TMTC | P6 | 30x107 | C6 | 26x106 | D6 | 48x106 |
| A7 | TMTC | P7 | 22x107 | C7 | 22x107 | D7 | 30x106 |
| A8 | TMTC | P8 | 52x107 | C8 | 28x107 | D8 | 21x106 |
| A9 | TMTC | P9 | 60x107 | C9 | 49x107 | D9 | 26x106 |
| A10 | TMTC | P10 | 58x107 | C10 | 47x107 | D10 | 38x106 |
| A11 | TMTC | P11 | 47x107 | C11 | 28x107 | D11 | 15x106 |
| A12 | 76x107 | P12 | 68x107 | C12 | 35x107 | D12 | 19x106 |
| A13 | 83x107 | P13 | 63x107 | C13 | 41x107 | D13 | 23x106 |
| A14 | 79x107 | P14 | 79x107 | C14 | 46x107 | D14 | 26x106 |
| A15 | 55x107 | P15 | 60x107 | C15 | 14x107 | D15 | 21x106 |

### 4.1.2 Total Coliform Count (TCC)

In this study, 5 samples (2 panipuri, 2 dahibada and 1 chatpatte) did not show presence of coliform whereas all the 15 Aalunimki samples gave positive result for coliform. In 3 aalunimki samples from Machavaudi had shown TMTC numbers of coliform. The lowest number of coliforms was found in dahibada sample. The highest coliform count was found to be 71x107cfu/g and the lowest TCC was found to be 3x106 cfu/g. (Table-2)

Table 2: Total Coliform Count

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sample Code | TCC(cfu/g) | Sample Code | TCC(cfu/g) | Sample Code | TCC(cfu/g) | Sample Code | TCC(cfu/g) |
| A1 | 56x106 | P1 | 5x106 | C1 | 19x106 | D1 | 16x107 |
| A2 | TMTC | P2 | Nil | C2 | 25x106 | D2 | 3x107 |
| A3 | TMTC | P3 | 7x106 | C3 | 21x106 | D3 | 21x107 |
| A4 | 61x106 | P4 | 9x106 | C4 | 26x106 | D4 | 15x107 |
| A5 | 47x106 | P5 | 4x106 | C5 | 16x106 | D5 | 16x107 |
| A6 | 65x106 | P6 | 10x107 | C6 | 10x106 | D6 | 24x107 |
| A7 | 71x106 | P7 | 7x107 | C7 | Nil | D7 | 31x107 |
| A8 | 46x107 | P8 | 8x107 | C8 | 25x107 | D8 | 14x107 |
| A9 | 11x107 | P9 | 56x107 | C9 | 31x107 | D9 | Nil |
| A10 | 13x107 | P10 | 44x107 | C10 | 23x107 | D10 | 23x107 |
| A11 | 46x107 | P11 | 35x107 | C11 | 21x107 | D11 | 14x107 |
| A12 | 56x107 | P12 | 65x107 | C12 | 18x107 | D12 | Nil |
| A13 | 68x107 | P13 | 47x107 | C13 | 29x107 | D13 | 21x107 |
| A14 | 60x107 | P14 | 58x107 | C14 | 31x107 | D14 | 20x107 |
| A15 | 47x107 | P15 | 55x107 | C15 | 12x107 | D15 | 18x107 |

(TMTC= Too Many to Count)

### 4.1.3 Occurrence of *Staphylococcus aureus*

Out of total 60 samples, *S. aureus* were found in 53% samples viz. 32 samples (7 aalunimki, 14 panipuri, 6 chatpate and 5 dahibada samples). The highest percentage of *S. aureus* was found in panipuri with 93.33% prevalence where as dahibada had the lowest prevalence of *S. aureus* with 33.33% occurrence. (Table 3)

**Table 3: Occurrence of *Staphylococcus aureus***

|  |  |
| --- | --- |
| Source Sample | *S. aureus* |
| Growth | Percentage |
| Aalunimki (n=15) | 7 | 46.66 |
| Panipuri (n=15) | 14 | 93.33 |
| Chatpate (n=15) | 6 | 40 |
| Dahibada (n=15) | 5 | 33.33 |
| Total (N=60) | 32 | 53.33 |

### 4.1.4 Occurrence of *E.coli*

Amongst 60 samples, *E. coli* were found in 67% samples viz. 40samples (11 aalunimki, 12 panipuri, 9 chatpate and 8 dahibada samples). The highest percentage of *E. coli* was found in panipuri with 80% prevalence where as dahibada had the lowest prevalence of *E. coli* with 53.33% occurrence (Table 4).

Table 4: Occurrence of *E. coli*

|  |  |
| --- | --- |
| SourceSample | *E. coli* |
| Growth | Percentage |
| Aalunimki (n=15) | 11 | 73.33 |
| Panipuri (n=15) | 12 | 80 |
| Chatpate (n=15) | 9 | 60 |
| Dahibada (n=15) | 8 | 53.33 |
| Total (N=60) | 40 | 66.67 |

### 4.1.5 Occurrence of *Salmonella spp*

Altogether, 36.67% samples (22 samples out of 60) showed positive result for *Salmonella* spp*.* Out of 15 dahibada samples, only two of them showed the presence of *Salmonella* but 53.33% aalunimki samples viz. 8 samples were found to be contaminated with *Salmonella*. (Table 5).

**Table 5: Occurrence of *Salmonella spp***

|  |  |
| --- | --- |
| Source Sample | *Salmonella* |
| Growth | Percentage |
| Aalunimki (n=15) | 8 | 55.33 |
| Panipuri (n=15) | 6 | 40 |
| Chatpate (n=15) | 6 | 40 |
| Dahibada (n=15) | 2 | 13.33 |
| Total (N=60) | 22 | 36.67 |

### 4.1.6 Occurrence of *Shigella* spp

Out of 60 samples, *Shigella* species werefound in only 11.67% samples viz. 7 samples. Among them, 3 samples were aalunimki, 3 samples were chatpate and 1 was panipuri sample. (Table-6)

Table 6: Occurrence of *Shigella species*

|  |  |
| --- | --- |
| SourceSample | *Shigella species* |
| Growth | Percentage |
| Aalunimki (n=15) | 3 | 20 |
| Panipuri (n=15) | 1 | 6.67 |
| Chatpate (n=15) | 3 | 20 |
| Dahibada (n=15) | 0 | 0 |
| Total (N=60) | 7 | 11.67 |

### 4.1.7 Occurrence of *Vibrio* spp

Amongst 60 samples, only 5 of them (2aalunimki, 2 panipuri, 1 chatpate) were found to be contaminated with *Vibrio* species. In this study, all dahibada samples were found to be free from *Vibrio* species. (Table-7)

Table 7: Occurrence of *Vibrio* spp

|  |  |
| --- | --- |
| Source Sample | *Vibrio species* |
| Growth | Percentage |
| Aalunimki (n=15) | 2 | 13.33 |
| Panipuri (n=15) | 2 | 13.33 |
| Chatpate (n=15) | 1 | 66.67 |
| Dahibada (n=15) | 0 | 0 |
| Total (N=60) | 5 | 8.33 |

### 4.1.8 Occurrence of *Bacillus cereus*

In this study, *Bacillus cereus* was isolated from 20% of total sample viz. 12 samples out of 60. Out of 15 aalunimki samples, 6 samples had shown the presence of *Bacillus cereus.* Similarly, 4 samples of panipuri and 2 of chatpate were contaminated with *B.cereus* (Table-8)

Table 8: Occurrence of *Bacillus cereus*

|  |  |
| --- | --- |
| Source Sample | *Bacillus cereus.* |
| Growth | Percentage |
| Aalunimki (n=15) | 6 | 40 |
| Panipuri (n=15) | 4 | 26.67 |
| Chatpate (n=15) | 2 | 13.33 |
| Dahibada (n=15) | 0 | 0 |
| Total (N=60) | 12 | 20 |

#### Relation of isolated organisms with Street food source

There was no significant relation between food sources with presence *S. aureu s* (P=0.501), food sources with presence of *E. coli* (P=0.527), food sources with presence of *Shigella* (P=0.304), food sources with presence of *Vibrio* (P=0.674) and food sources with presence of *B. cereus* (P=0.708) but significant relation between food sources and presence of *coliform* (P=0.048). (Table-9)

Table 9: Relation of isolated organisms with food source

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SourceOrganism | Aalunimki(n=15) | Panipuri(n=15) | Chatpate (n=15) | Dahibada(n=15) | Chi square test Source verses Organism |
| Total coliform | 15 | 14 | 14 | 13 | P=0.048 |
| S. aureus | 7 | 14 | 6 | 5 | P=0.601 |
| E. coli | 11 | 12 | 9 | 8 | P=0.426 |
| Salmonella | 8 | 6 | 6 | 2 | P=0.057 |
| Shigella | 3 | 1 | 3 | 0 | P=0.548 |
| Vibrio | 2 | 2 | 1 | 0 | P=0.474 |
| B. Cereus | 6 | 4 | 2 | 0 | P=0.802 |

## 4.2 Antibiotics Susceptibility Pattern of Isolates

All the isolated bacterial pathogens (32 *S. aureus*, 40 *E. coli*, 22 *Salmonella*, 7 *Shigella*, 5 *Vibrio* and 12 *B. cereus)* were subjected to the antibiotic susceptibility test to the 8 different antibiotics namely Amoxycillin, Azithromycin, Amikacin, Cefoxitine, Naldixic acid, Ciprofloxacin, Cotrimoxazole, Oflaxacin, Tetracycline and Chloramphenicol. All of the isolates were resistant to Amoxycillin.

### 4.2.1 Antibiotic Susceptibility Test of *S. aureus*

All of the isolated *S. aureus* were resistant to Amoxicillin. Most of the S*. aureus* (93.75%) were sensitive to Amikacin and 62.5% of them were sensitive to Azithromycin, 53.13% to Cifotoxime, 34.34% to Nalidicacid and 71.88% to Ciprofloxacin. 9.41% of them were resistant to Nalidixic acid, 59.38% to Tetracycline, 12.5% to Cefotoxime and 5.67% to Chloramphenicol. (Table 10)

Table 10: Antibiotic Susceptibility Test of *S.aureus*

|  |  |
| --- | --- |
| Antibiotics Used | *S. aureus*(n=32) |
| S (%) | I (%) | R (%) |
| AM | 0 | 0 | 100 |
| AZM | 62.5 | 37.5 | 0 |
| AK | 93.75 | 6.25 | 0 |
| CX | 53.13 | 34.37 | 12.5 |
| NA | 34.34 | 56.25 | 9.41 |
| CIP | 71.88 | 28.12 | 0 |
| T | 9.38 | 31.24 | 59.38 |
| C | 14.70 | 79.63 | 5.67 |

S= Sensitive, I= Intermediate sensitive, R= Resistant

### 4.2.2 Antibiotic Susceptibility Test of *E. coli*

All of the *E.coli s*howed resistance against Amoxycillin. 92.5% *E.coli* were resistant to Tetracycline, 25% resistant to Nalidixic acid and 20% to Ciprofloxacin. Similarly, 97.5% of *E.coli* was sensitive to Amikacin, 40% to Azithromycin, and 27.5% to Nalidixic acid. (Table 11)

Table 11: Antibiotic Susceptibility Test of *E.coli*

|  |  |
| --- | --- |
| Antibiotics Used | *E. coli* (n=40) |
| S (%) | I (%) | R (%) |
| AM | 0 | 0 | 100 |
| AZM | 40 | 60 | 0 |
| AK | 97.5 | 2.5 | 0 |
| CX | 0 | 80 | 20 |
| NA | 27.5 | 47.5 | 25 |
| CIP | 81.48 | 18.51 | 0 |
| T | 0 | 7.5 | 92.5 |
| C | 22.22 | 77.77 | 0 |

S= Sensitive, I= Intermediate sensitive, R= Resistant

### 4.2.3 Antibiotic susceptibility test of *Salmonella spp*

In case of *Salmonella,* all of the isolates were immediately sensitive to Amikacin, Azithromycin, Cefotoxime and Ciprofloxaciin. But 17.86, 28.57 and 10.71% of *Salmonella* were resistant to Nalidixic acid, Tetracycline and Chloramphenicol respectively. (Table 12)

Table 12: Antibiotic susceptibility test of *Salmonella*

|  |  |
| --- | --- |
| Antibiotics Used | *Salmonella* (n=22) |
| S (%) | I (%) | R (%) |
| AM | 0 | 0 | 100 |
| AZM | 28.57 | 71.43 | 0 |
| AK | 64.29 | 35.71 | 0 |
| CX | 42.86 | 57.14 | 0 |
| NA | 28.57 | 53.57 | 17.86 |
| CIP | 53.57 | 46.43 | 0 |
| T | 0 | 71.43 | 28.57 |
| C | 21.43 | 67.86 | 10.71 |

S= Sensitive, I= Intermediate sensitive, R= Resistant

### 4.2.4 Antibiotic susceptibility test of *Shigella* spp

100% *Shigella* were sensitive to Ciprofloxacin and 100% resistant to Amoxycillin. 71.43% of them were found to be resistant to Tetracycline and 28.57% showed resistance to Chloramphenicol. Most of the *Shigella* spp were intermediate sensitive to antibiotics used. (Table-13).

**Table 13: Antibiotic susceptibility test of *Shigella***

|  |  |
| --- | --- |
| Antibiotics Used | *Shigella*(n=7) |
| S (%) | I (%) | R (%) |
| AM | 0 | 0 | 100 |
| AZM | 28.57 | 71.43 | 0 |
| AK | 28.57 | 71.43 | 0 |
| CX | 71.43 | 28.57 | 0 |
| NA | 28.57 | 71.43 | 0 |
| CIP | 100 | 0 | 0 |
| T | 0 | 28.57 | 71.43 |
| C | O | 71.43 | 28.57 |

S= Sensitive, I= Intermediate sensitive, R= Resistant

### 4.2.5 Antibiotic susceptibility test of *Vibrio*

All of the *Vibrio* spp were intermediate sensitive to Azithromycin whereas, 60% of them were intermediate sensitive to Amikacin, Nalidixic acid and Tetracycline. 100% *Vibrio* spp were found to be resistant towards the Amoxycillin. Similarly, 40% Vibrio were resistant to Tetracycline and Chloramphenicol. (Table 14)

Table 14: Antibiotic susceptibility test of *Vibrio*

|  |  |
| --- | --- |
| Antibiotics Used | *Vibrio* (n=5) |
| S (%) | I (%) | R (%) |
| AM | 0 | 0 | 100 |
| AZM | 0 | 100 | 0 |
| AK | 40 | 60 | 0 |
| CX | 20 | 80 | 0 |
| NA | 40 | 60 | 0 |
| CIP | 60 | 40 | 0 |
| T | 0 | 60 | 40 |
| C | 20 | 40 | 40 |

### 4.2.6 Antibiotic susceptibility test of *Bacillus cereus*

100% of *B. cereus* was resistant to Amoxycillin, 58.33% of *B.cereus* was sensitive to Ciprofloxacin and 25% of them were resistant to Tetracycline and 8.33% resistant to Chloramphenicol. Comparatively, more resistivity was seen in Tetracycline and Chloramphenicol. No resistivity was seen against Azithromycin, Amikacin, Cefotoxime, and Nalidixic acid. (Table 15)

Table 15: Antibiotic susceptibility test of *Bacillus Cereus*

|  |  |
| --- | --- |
| Antibiotics Used | *B. cereus*(n=12) |
| S (%) | I (%) | R (%) |
| AM | 0 | 0 | 100 |
| AZ | 41.67 | 58.33 | 0 |
| AK | 25 | 75 | 0 |
| CX | 58.33 | 41.67 | 0 |
| NA | 66.67 | 33.33 | 0 |
| CIP | 58.33 | 41.67 | 0 |
| T | 16.67 | 58.33 | 25 |
| C | 25 | 66.67 | 8.33 |

S= Sensitive, I= Intermediate sensitive, R= Resistant

All isolates showed resistance to at least two or three antimicrobials. Resistance to a wide range of antibiotics was observed. Resistance rates to amoxicillin, tetracycline, and chloramphenicol were higher compared to other antibiotics.

# List of Photographs

|  |  |
| --- | --- |
| **IMG20170530172414.jpg****Working on Lab** | 110.jpg**Enrichment of the Sample** |
| 111.jpg **Growth of Vibrio in TCBS** | 11 (1).jpg**AST of Isolates** |
| 22.jpg**AST of Isolates** | **33.jpg****AST of Pseudomonas** |

**Biochemical Tests**

# CHAPTER V

# DISCUSSION

Street foods are favorable growth media for microorganisms including many pathogens because of their high moisture content. Any food to be of good quality should be free from hazardous microorganisms. Mainly the young generations are attracted towards the street food because of its variation and taste. The increasing population, urbanization, and the modernization of the Dharan are also responsible for the pollution. The impact of pollution is also on various food borne diseases due to contamination by various pathogenic bacteria. Microorganisms set into the food products by water, unclean utensils, knives, environmental contamination and handling of food in its preparation and sales. Due to lack of scientific methods of storage and due to lack of knowledge of microorganisms, many types of microorganisms introduce into the street food. Once microorganisms are introduced into the food, they multiply rapidly and reach levels sufficient to produce infections or intoxications depending upon the types of invasion.

The number of microbes in food at any given time depends on its handling, storage condition, storage temperature and length of time it has been kept. The contaminating organisms may include those responsible for food borne illness. But the number of dose of organisms necessary to infect or to produce sufficient toxin to cause symptoms not only varies with the species and kind of organisms but also varies with the resistance of the person who consumed the food products. Even though the microbial population in the food does not cause food borne disease, certain microbial contamination is an indicator of poor sanitary practice in the processing and storage of such street food.

In this study table-1 revealed that Total Plate Count were TMTC in 9 aalunimki samples 2,3,4,6,7,8,9,10 and 11.The highest TPC was found in Aalunimki sample 13(83×107 ) and the lowest TPC was found in sample Chatpate 15(14×107).The highest load of bacterial pathogens were found in area while the lowest bacterial load was found. The microbiological condition of street food of Dharan is found to be heavily contaminated with pathogenic organisms, keeping in mind the unhygienic outlets and lack of microbiological standard of street food.

In the case of Total Coliforms Count, samples (Panipuri 2, Chatpate-7 D-9 and D-12 ) did not show presence of coliforms whereas all the 15 samples of aalunimki gave positive result for coliforms. In 5 aalunimki sample from Bargachii to Bhanuchowk had shown TMTC numbers of coliforms. The lowest number of coliforms was found in sample. The highest coliform count was found to be .The presence of coliform in food indicates contamination and possibility of presence of harmful organisms, total coliform counts were used as an indicator of the overall hygiene status of food products (Maddigon et al., 2000)The result obtained in this study is higher than previous researches indicating increasing unhygienic practices, which is more illustrated by following studies.

In the previous study of Karki (2005) the average total plate counts were found to be 4.31×104, 8.38×104, 2.62×102 and 1.35×104cfu/g in *chatpate*, *dahibada*, *momo* and *panipuri* samples respectively, collected from various food shops of Biratnagar sub-metropolitan city. Also, the average total coliform counts were 1.70×104, 6.77×103 and 1.35×104cfu/g in *chatpate*, *dahibada* and *panipuri* samples respectively.

 According to the work previously performed by, Dahal (1993) found that the total plate counts in *chatpate* and *dahibada* samples collected from Dharan markets were in the range of 5.7×106 to 1.1×108 and 1.8×107 to 1.2×108cfu/g respectively, whereas coliform counts in the same were in the range of 1.0×104 to 9.3×104 and 1.0×104 to 9.0×104cfu/g respectively (Appendix F). It shows that the hygiene of street foods is very poor in Nepal as a whole.

In this study, the total plate count was found to be higher in *chatpate, panipuri* and *dahibada* than reported Karki (2005). Whereas, it was lower in *chatpate* and *dahibada* than reported by Dahal (1993). Similarly, the presence of coliform was found to be higher in *dahibada* and lower in *chatpate* and *panipuri* than reported by Karki (2005). In *chatpate* and *dahibada*, total coliform count was lower than reported by Dahal (1993).Taking the reference of ICMSF guidelines for assessment of microbiological quality of ready-to-eat foods at point of sale (Appendix B), the average total plate count was found to be satisfactory for all samples. The average total coliform count of the *dahibada* samples was found to be satisfactory, whereas the same for the remaining samples analyzed were unsatisfactory. Street foods containing large numbers of bacteria do not present a health hazard, but it should be viewed as having been produced unhygienically or poorly stored or contaminated during processing.

The result presented in the above table- to table- revealed the pathogenic species isolated in this study were *S.aureus, E. coli, Vibrio spp., Salmonella spp, shigella spp. And B.Cereus* which is comparable to the study conducted in Harare (FAO/WHO2005).

In this study, *S.aureus* were found in 53.33% samples viz samples ( aalunimki, Panipuri, Chatpate and Dahibada). The highest percentage of *S.aureus* was found in Panipuri sample with 93.33% prevalence where as Dahibada has lowest prevalence of *S.aureus* with 33.33% occurrence (Table-3). This prevalence is higher than previous researches.

The studies on street vended food in Few African countries, Asia and USA, have revealed high bacterial counts and presence of foodborne pathogens (Mosupuye& Von Holy,1999: Bryan et al., FAO/GHANA, 1997). However, the information of street food quality is not readily available in developing countries.

(Table10) showed the AST pattern of isolated *S.aureus* showing most of the *S.aureus* ( 93.75%) were sensitive to Amikacin and 62.5% of them were sensitive to Azitromycin, 34.34% to Nalic acid,9.38% to Tetracyline, 14.70% to Choramphenicol ( Table -10 ).In average 22.79% *S.aureus* showed multidrug resistance which is greater than the study by Datta et al (2012).

The result shown in table no. showed that *Salmonell spp*. was found in 53.33% aalunimki, 40% Panipuri, 40% Chatpate, and 13.33% Dahibada. It is higher than the previous studies.by (Sheth et al., 2005). This showed the unhygienic condition of food stall and utensils used and poor sanitation of street food. In the previous study of (Sheth et al.,2005) *Salmonell*a and *Shigellaspp* were most likely to found in knife,hand rinse and dish water samples.

In case of *Salmonella* 17.86%, 28.57% and10.71% of *salmonella* were resistant to Nalidixicacid,Tetracycline and Cholramphenicol respectively (Table 12). The antibiotic susceptibility pattern of *salmonella* was almost similar to the result of (Dhakal Laxman et al.,2016).

*Shigella* species were found in only 11.67% of samples viz 3 samples of Aalunimki,3 samples of Chatpatte and 1 was panipuri sample.100% *Shigella* were found sensitive to ciprofloxacin and 100% resistant toAmoxycillin. 71.43% was found resistant to Tetracycline and 28.57% showed resistant to Choramphenicol. Most of the them were intermediate sensitive to antibiotics used. *Vibro spp* were found in 8.33% food sample viz 2 aalunimki,2 Panipuri,1 Chatpate whereas *Vibrio spp.*was not isolated from any Dahibadasample.All of the *Vibrio spp* were intermediate sensitive to Nalidixic acid. Likewise, 40 % of them were found sensitive to Amikacin, 60% to Ciprofloxacin,100% *Vibrio spp* were found resistant to Amoxycillin.

In this study, *B. cereus* was isolated from 20% of food samples viz 6 aalunimki,4 panipuri and 2 chatpate sample whereas*B.cereus* was not found in dahibada..The presence of *B.cereus* in the food sample indicates that the food is either contaminated by the dust and pollution or it may be due to cross contamination from raw ingredient mixed in street food. In the AST (Table-15) showed that100% *B.cereus* was resistant to Amoxycillin, 58.33% were sensitive to Ciprofloxacin and 25% was resistant to Tetracycline.

Presence of *E.coli*, indicator of hygiene and sanitary quality, suggests that consumers are at high risk of being food poisoned and other pathogenic organisms. In this study of 60 samples. *E.coli* was found in 67% of food sample viz sample (11 aalunimki, 12 panipuri, 9 chatpate and 8 dahibada samples).The prevalence is higher than the previous studies. All the isolated *E.coli* of this study showed resistivity against Amoxycillin.40% were sensitive to Azitromycin, 81.48% to Ciprofloxacin,97.5% to Amikacin (Table 11).

All the isolates showed resistance to at least two or three antibiotics. Resistance rate to Amoxycillin, Tetracycline were higher. Presence of multi drug resistance *Salmonella* in a ready to eat food sample is an alarming challenge to public health issue.

From the statistical analysis, there was no significant relation between food source with the presence of coliforms (P=0.048) with the presence of *E. coli* (P=0.426), *S.aureus* (P=0.601), *B. cereus* (P=0.802),with the presence of *Shigella (*p=0.548) but significant relation between food source and presence of *salmonella* (P=0.057).The higher number of organism obtain in this study might be due to the contaminating source, use of raw vegetable, dust, knifes and unsterilized utensils. Cross contamination cannot be ignored. The presence of these microbes in food can be linked to a number of factors such as improper handling and processing, use of contaminated water during washing and dilution, cross contamination from rotten fruits and vegetables, or the use of dirty processing utensils like knife and trays (Bryan et al*.*, 1992; Khalil et al*.*, 1994).

# CHAPTER VI

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

Microbiological analysis of street food of Dharan was assessed and following conclusion can be drawn from the study. All the food samples were found to contain higher microbial load than prescribed standards. The bacterial counts of food samples were found to be high which might be due to poor sanitary condition of street shop, handlers and premises. This study reveals the unhygienic practice of food handlers, poor sanitation of stall. Most of the Street food vendors lack basic education. Presence of multi drug resistant *salmonella spp*. in the ready to consume food is the great challenge to public health issue, which need to be studied in larger context and figure out the source of contamination quickly. The result of AST revealed that all the isolated pathogens were resistant to two or three subjected antibiotics Resistance rate of Amoxicillin was higher. The finding suggests about the unscientific method of handling, lack of information about sanitation resulting higher degree of contamination. In almost all the food samples the Total Plate Counts and Total Coli form counts were surprisingly higher which indicates inadequate cleanliness, Unsanitary handling, poor condition of stalls/shops, handling and processing premises. Consumption of such food may be hazardous to human health.

## 6.2 Recommendations

To improve the microbiological quality of street food, following recommendations can be taken out:

1. Proper hygiene and sanitation to be maintained by food handlers in properly constructed hygienic surrounding (cartwheel) by improved methods.
2. Food vendors should be encouraged to operate from designated environmentally sound places.
3. The act of Food legislation and guidelines should be developed to recognize the street food industry by developing code of practice for street food vending.
4. First-In-First-Out (FIFO) approach should be implemented for both quality and safety reasons. FIFO means that the first batch of the product prepared should be sold first. The FIFO concepts minimize the pathogens growth, cross contamination, and encourage product rotation.
5. Food handler, Street food vendor or helper should be trained in all issues regarding personal cleanliness, environment sanitation and hygienic food processing.
6. Awareness campaigns regarding the food safety and relation to public health should be carried out through the radio, television, posters and billboards.
7. Concerned authority should check and monitor the quality of street foods strictly implemented for public health protection.
8. Licensing of street food vendors should be done, which aids as communication channel between the municipal officials, health inspectors and food vendors.
9. Consumers should also be alert about the requirements for healthy and safe food, especially street vended foods.
10. Vendors and helpers suffering from communicable disease should not be involved in food preparation and handling.

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