ASSESSMENT OF THE MICROBIOLOGICAL SAFETY IN STREET FOOD VENDING IN LOCAL MARKET OF ITAHARI MUNICIPALITY

by

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Assessment of the Microbiological Safety in Street Food Vending in Local Market of Itahari Municipality

A dissertation submitted to the Food Technology Instruction Committee in Tribhuvan University in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This *dissertation* entitled *Assessment of the Microbiological Safety in Street Food Vending in Local Market of Itahari Municipality* presented by Sandesh Paudel has been accepted as the partial fulfillment of the requirements for the B. Tech. in Food Technology.

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(Mr. Sandesh Paudel)

Abstract

A study to assess the microbiological status of street foods (ready-to-eat foods and beverages) sold in local market of Itahari municipality was carried out. Eight different samples of each six various street foods along with eight water samples were collected and analysed. Microbiological analysis revealed the average TPC to be $3.00x10^2$, $6.22x10^5$, $1.73x10^5$, $4.62x10^3$, $7.44x10^3$ and $6.51x10^5$ cfu/g in *momo*, *chatpate*, *panipuri*, *aluchop*, *pyaji* and *dahibada* samples respectively. Similarly, the total coliform counts were $1.64x10^1$, $1.14x10^4$, $8.14x10^3$, $3.78x10^2$, $9.03x10^2$ and $9.08x10^3$ cfu/g in *momo*, *chatpate*, *panipuri*, *aluchop*, *pyaji* and *dahibada* samples respectively. *Salmonella* was not detected in any of the samples. The average value for the total plate count and total coliform count of water samples were found to be $1.28x10^2$ and $3.74x10^1$ cfu/g respectively. Microbiological analysis also showed that 8.3% of samples had unsatisfactory quality in total plate count and 89.6% in coliform count. There were significant differences in microbial load within the samples and sample types collected from different locations, indicating the hygienic practice (keeping other things constant) in the street food stalls being significantly different.

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Abbreviations

A.D.	Anno Domini
Anon	Anonymous
ANOVA	Analysis of Variance
CAC	Codex Alimentarius Commission
CBS	Central Bureau of Statistics
CCT	Central Campus of Technology
cfu/g	Colony forming units per gram
CNN	Cable News Network
DFTQC	Department of Food Technology and Quality Control
et al.	and others
FAO	Food and Agricultural Organization
HACCP	Hazard Analysis and Critical Control Point
hrs	Hours
ICMSF	International Commission on Microbiological Specifications for Foods
IM	Itahari Municipality
ng	Nanogram
NHRC	Nepal Health Research Council
p.m.	Post Meridian
ppm	Parts per million
TPC	Total Plate Count
US$	United States Dollar
WHO	World Health Organization

Part I

Introduction

1.1 General Introduction

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 who are either stationary or ambulatory. The definition also includes fresh fruits and vegetables which are sold outside authorized market areas for immediate consumption.

Street foods represent a significant part of urban food consumption for millions of low-and-middle-income consumers, in urban areas on a daily basis. 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. Street foods have become increasingly popular all over the world and Nepal is no exception to it. The street foods have grown both in volume as well as varieties to cater to the variety of tastes. In developing countries, street food preparation and selling provides a regular source of income for millions of men and women with limited education or skills.

Itahari municipality (4177 hectare area) is a town in Sunsari district in the Koshi zone of south-eastern Nepal. It is located at the main transportation junction of eastern Nepal. It is the center of the East-west Mahendra highway and North-south Koshi highway and thus is a town of emerging importance. The population is increasing rapidly (at 4.39% annual growth rate) resulting in an increasing problem with regard to sanitation, hygiene, availability of clean water and food hygiene (IM, 2007). 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.

In the past, people have been consuming only home-made foods, but nowadays people have been attracted towards the hotel, restaurants and street foods. The food habit among

the people at Itahari has vast demarcation due to the various groups of people and also their busy life. Street foods are mainly found in the town rather than the village. These are mainly found where population gathering occurs such as near school, picture hall, bus stop, chowk and main market. The street foods are mainly consumed by population of age group 10-35 years and less commonly by other age groups. 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 in the quality of street foods. Some of the commonly occurring diseases due to contamination are diarrhea, dysentery, cholera, fever, etc. Consumers are also responsible for this deteriorating condition. According to WHO (2002), 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. And a common mistake committed by most consumers is blindly picking up the product without giving a second thought to its hidden defects.

Although the potential health risk originating from street foods is of great public health concern to the authorities but it is well recognized that this informal sector has significant positive impact on the socio-economic situation of the city. The major concern is related to food safety, but other concerns are also reported, such as sanitation problems (waste accumulation in the streets and the congestion of waste water drains), traffic congestion in the city also for pedestrians (occupation of sidewalks by street vendors and traffic accidents), illegal occupation of public or private space, and social problems such as child labor, unfair competition to formal trade, etc. (FAO, 2003).

1.2 Justification of the work

Evaluation of quality before consuming is a must for any food (Ghosh, 2007). The street foods 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 (FAO, 2003). Both the street food venders and consumers of Itahari are not very sensitive towards the food quality. Although 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 Itahari are safe. Due to this, an investigation is necessary to assess the safety of street foods.

1.3 Objectives

1.3.1 General objectives

The general objective of my study is to assess the food safety in street foods endorsing their microbiological aspects.

1.3.2 Specific objectives

a. To find out the extent of microbial contamination to see whether the street foods are safe or not for human consumption.

b. To enumerate the indicator/index organisms and pathogenic flora of street foods, e.g. *Salmonella*, coliforms, etc.

c. To study the microbiological quality of water used by street food vendors.

1.4 Limitations

a. A wide variety of street foods are available in the market of Itahari municipality, but only few commonly consumed foods were considered for the examination and the results were generalized by analyzing data collected from selected places.

b. During the period from sampling to test and analysis, there was a chance of possible growth of micro-organisms which can affect the results.

c. The study was limited to the investigation of some common pathogenic bacteria like *Salmonella* and indicators like coliform only, because of time and materials constraints.

d. The study was carried out only during summer season and the microbial load is not similar in all seasons.

1.5 Expected output

The thesis moves around the following microbiological analysis and examination: total plate count, coliform count and *Salmonella* count. The present work will generate adequate data giving true picture about the microbial contamination of street foods vended in Itahari.

Upon analysis of the data, the work will be able to recommend techniques and ideas for municipality for providing safe and wholesome street foods 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.

Part II

Literature Review

2.1 Street foods

Street foods are defines 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).

The central characteristic of street foods in this definition is their retail location, i.e. "on the street." To differentiate street food vendors from formal sector food establishments, such as restaurants, street foods are further qualified as being sold on the street from "pushcarts or baskets or balance poles, or from stalls or shops having fewer than four permanent walls". In terms of production, street foods may be centrally processed foods made by the formal sector food industry, or they may be processed within the street food trade either by the vendor her/himself or another small-scale processor (Draper, 1996).

Street foods are ready-to-eat foods or beverages, which include many types of foods ranging from cereal and fruits to cooked meats and drinks. They are usually sold in busy public areas, such as pavements, roadways, back alleys of markets, school premises, bus and railway stations, beaches, parks and other public places. They are served with the minimum amount of fuss in individual portions dished into take-away containers, such as disposable plastic, paper, styrofoam plates, bowls, cups and utensils (Anon, 2008).

2.1.1 Some common street foods

Street foods represent a significant part of urban food consumption for millions of low-and-middle-income consumers, in urban areas on a daily basis. Street foods have become increasingly popular all over the world and Nepal is no exception to it. Street foods have grown both in volume as well as variety to cater to the variety of tastes. As concerned to Itahari municipality, some commonly sold street foods are:

a. *Momo*: It is a type of dumpling that originated from Tibet. *Momos* are made with simple flour-and-water dough. The dough is fashioned into small circular flat pieces. The filling is then enclosed either in a round pocket or in a half moon shape or crescent. The dumplings are then cooked by steaming. The filling may be one of

several mixtures including minced meat, vegetables (cabbage, etc), cheese and so on. In Nepal, buffalo, goat, chicken and pork meat are very popular (Wikipedia, 2010a).

b. *Chatpate*: It is a compounded product which contains the mixture of puffed rice, onions, tomato, salt, chili powder, oil, lemon juice 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 (Wikipedia, 2010b).

c. *Panipuri*: It is also known as *golgappa*, *puchka* and *gup chup*. 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. It is small enough to fit completely in one's mouth. The word p*ani* comes from the Nepali/Hindi word for water and *puri* is the bread made by frying dough in oil (Wikipedia, 2010c).

d. *Alu chop*: It is a popular food made from potato. Potatoes are boiled and mashed. The other ingredients mixed during mashing are salt, chili powder, chopped onion, chopped ginger and chopped green chili. Water may be added to make thick paste. It is then divided into small parts, made into balls and pressed flat. Each piece is dipped into besan (chick pea flour) batter/paste and shallow fried in oil for a few minutes until golden brown on both sides (Anon, 2010a).

e. *Pyaji*: Onion pakora is known as *pyaji* in Bengali. It is one of a kind mouth-watering snack. Chopped onions, green chilies and fresh coriander leaves are taken first in a big bowl. The other ingredients, viz. besan, salt and red chili powder are then added and mixed together. Water is added slowly as needed to make a smooth creamy batter, not runny. A big spoonful of batter is dropped into hot oil in a pan and shallow fried in oil for a few minutes until dark brown on both sides (Anon, 2009a).

f. *Dahibada*: It is a popular South Indian dish. It consists of dahi (yoghurt) and *bada*. For *bada*, clean *urad daal* is taken, washed and soaked overnight and then grinded into smooth paste. Salt is added for taste. Oil is heated in a pan and spoonfuls of batter are dropped and fried till golden brown. Hot *badas* are put in cold water for 2-3 minutes and then water is squeezed. For dahi, it is 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 foods alleviate 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, colour and appearance). The nutritional value of street foods depends upon the ingredients used and how they are prepared, stored and sold (Chakravarty and Chittranjan, 1995).

Eating of a combination of street foods provide the consumer the adequate opportunity to meet his or her daily nutritional requirements at an affordable pace. A study in Kolkata found that an average 1000 calorie meal contained about 30 gm protein, 15 gm fat and 180 gm carbohydrates. It was reported that average energy content of street foods ranges from 5-679 calories per 100 grams. It was observed that the street foods could provide 200 calories of energy per Re. 1/- (0.02 US$), which works out to be approximately 1000 calories for Rs. 5-6. It is estimated that the recommended daily energy intake can be met by consuming street foods which cost approximately US$ 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

The informal sector street food industry employs large number of people, both directly and 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. In Bangkok, 90% of the populations eat most of their meals outside the home (Dawson and Canet, 1991). In Bangkok, Thailand, 20,000 street food vendors provide city residents with 40% of their overall energy intake (FAO, 2001).

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:

- Street foods were accessed because they were convenient and affordable to the lower and middle-income groups. Almost 90% of street food consumers were men.
- Street foods can be classified into the following five categories on the basis of sales: meals and side dishes, snacks, sweets, frozen foods & drinks and miscellaneous foods.
- 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.
- All the samples were contaminated by one or more pathogenic organisms, especially *E. coli*, *Klebsiella*, *Proteus*, etc.

The world is urbanizing at a rapid pace. By 2015 A.D., it is estimated that 16 of the world's 26 cities with populations of 10 million or more inhabitants will be in Asia. 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. Consequently issues of food quality, food safety and environmental health, disposal of food waste, etc are of great safety concerns. 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, which make ideal breeding grounds for water and food-borne bacteria. The unemployed, new migrants, single mothers with dependent children, disabled or old

people, indigenous people, ethnic minorities, formal sector workers with declining or unstable incomes and those dependent on "crowded" informal sector activities, are some of the most vulnerable urban groups exposed to the health risks of urban food retailing.

Unbridled growth in the number of street vendors, food joints, hostels, fast food corners, catering services, canteens, cafeterias and restaurants as urban food catering services has given rise to grave food safety concerns. Problems can be caused by a variety of factors in preparation and handling (including cleaning, sorting and grading of food items); in packaging (to facilitate handling, promote hygiene and pack into small units); in storage (such as cold storage and refrigeration facilities, which are often insufficient, badly managed or too expensive) and in vending. Water, critically needed for processing, cooking, drinking water and sanitation, is getting scarcer in urban setting both in terms of availability and quality (Ghosh, 2007).

Foodborne diseases are common in most countries of the south-east Asia region. A large number of people suffer from communicable diseases caused by contaminated food, including drinking water, which can be a major cause of cholera and other forms of epidemic diarrhoeal diseases. Foodborne diseases are one of the major causes of malnutrition. The increasing use of chemicals in agriculture and in food processing industries has added new health concerns resulting from chemical contamination of food. While several countries in the region have food legislation, many lack well-defined national food safety policies and strategies (WHO, 2001).

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 on to many customers (FAO, 2001).

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 are 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).

The hygienic aspects of street food vending are a major concern for food quality control officers. Vending stands are often crude structures, and running water, washing facilities and toilets may not be available. Previous studies in countries such as Bangkok, Nigeria, Zambia, Calcutta and various Latin-American countries, have all indicated that food safety and quality are of particular importance in street-vended foods. Whilst the importance of street vended foods is not to be negated as an important source of nutrition as well as income and work opportunities offered for the informal sector, the safety of the foods is a particular problem, especially from microbiological point of view (Bryan *et al*., 1997).

2.2.1 Microbiological safety

Food borne illness of microbial origin is a major international health problem associated to food safety and an important cause of death in developing countries (WHO, 2002). 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. *Shigella sonnei* 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*. In Senegal, more than 200 cases of food poisoning were reported and street foods made from dairy products were incriminated (Dawson and Canet, 1991). In Cuba, 14 people died and 70 others were hospitalized for food poisoning after eating fried foods sold by a private vendor (CNN, 1999).

2.2.2 Heavy metals and pesticide residues

Due to the conditions under which street foods are sold, there is concern that food may be contaminated by heavy metals and pesticide residues. These contaminants may come from the utensils, raw materials, or transport methods used and may also occur due to the lack of appropriate storage facilities.

A study carried out in Accra revealed that street food vendors source their pots and other utensils from both formal and informal manufacturers/retailers. Some of the street food samples had higher levels of lead, cadmium, arsenic, mercury and copper than average food samples, suggesting possible leaching from the utensils (Tomlins *et al*., 2004).

2.2.3 Artificial coloring matters

Artificial colors are sometimes added to improve the appearance and eye appeal of the preserved food products. Food vendors also colour their products using dyes meant for cloth and paper. 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).

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).

2.2.4 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).

2.2.5 Environmental hygiene

Vendors usually congregate in overcrowded areas where there are high numbers of potential customers. Such areas usually provide limited access to basic sanitary facilities such as running water, garbage disposal and clean toilets. In these areas large amounts of garbage accumulate and provide harbor for insects and animal pests. Such conditions have given rise to many concerns regarding the sanitary standards of street-vending operations, especially because consumers are concerned about the price of food rather than its safety and hygiene in many cases.

Inadequate refuse disposal facilities lead to the accumulation of refuse at food vending sites. This leads to an increased pest population and will result in an increased risk of food contamination. In most instances, refuse collection are not available. At the same time, the vending operations being illegal, vendors contribute nothing towards the maintenance of infrastructure or provision of public services. This contributes to further deterioration of the hygienic condition of the area where the foods are vended.

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 Food microbiology

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. Many of our foods support the growth of pathogenic microorganisms or at least serve as a vector for them (Fraizer and Westhoff, 2008a).

2.3.1 Sources of contamination

Growing plants carry a typical flora of microorganisms on their surfaces and may become contaminated from outside sources. Animals likewise have a typical surface flora plus an intestinal one, give off organisms in excretion, and also become contaminated from outside sources. Plants and animals with parasitic disease carry the pathogen causing the disease. The inner, healthy tissues of plants and animals are reported to contain few living microorganisms or none (Fraizer and Westhoff, 2008b). The fruit or vegetable is harvested, milk is drawn, eggs are gathered, fish and other products are obtained from natural waters, and animals are collected and slaughtered, all carrying contaminating microorganisms from natural sources. In most instances, with the start of human handling, further contamination begins and it continues while the product is being handled and processed (Fraizer and Westhoff, 2008c).

Food products compounded from combinations of different food groups also would combine their microbial contents, and the new product may furnish a good culture medium for microorganisms that previously had little chance to grow. For e.g., yeasts from sugar added to bottled soft drinks may spoil the product. The water and flavoring materials are also potential sources of contamination. Spices and other condiments added to foods may be important sources of microorganisms. Microorganisms are added to compounded foods by ingredients such as spices, condiments, eggs and pickles. Salt, especially solar salt, may add halophilic and salt-tolerant bacteria to salted or brined products (Fraizer and Westhoff, 2008d).

Bacteria, molds and viruses are widely distributed. They may be found in water, soil, air and other substances which provide the proper conditions for growth and proliferation. Food serves as a vehicle and means of transmission in the final link of the chain of infections. The role of food here is significant since the product may not only permit the survival of the pathogens, but also may also provide a suitable medium for the rapid proliferation of micro-organisms and the production of toxin (WHO, 1996).

According to FAO/WHO (2003), 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. It is not only prominent but the inability of maintenance of proper hygienic conditions in and around street foods to be more prone to microbial contamination. Major factors leading to microbial contamination of street food are as follows:

a. Lack of basic infrastructure and services, such as potable water supplies.
b. Difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature.
c. Insufficient resources for inspection and laboratory analysis.
d. General lack of factual knowledge about the microbiological status or the precise epidemiological significance of many street-vended foods.
e. Poor knowledge of street vendors in basic food safety measures.
f. Inadequate public awareness of hazards posed by certain street foods.

2.3.2 Street-vended foods contamination mechanism pathways

Fig. 2.1 shows the causes, routes and vectors of various contaminations of street-vended foods. Four main vectors can be distinguished. The first vectors include insects and animals, the second is constituted by environmental conditions (weather, dust, rains, winds, urbanization), the third vectors include peoples acting in street food areas (government and his specific services low actions, hygiene controllers producers, growers, transporters, consumers and vendors). Finally, the last vector is represented by natural contaminants as toxins contain in some raw foodstuffs and sea foods (Nicolas *et al.*, 2007).

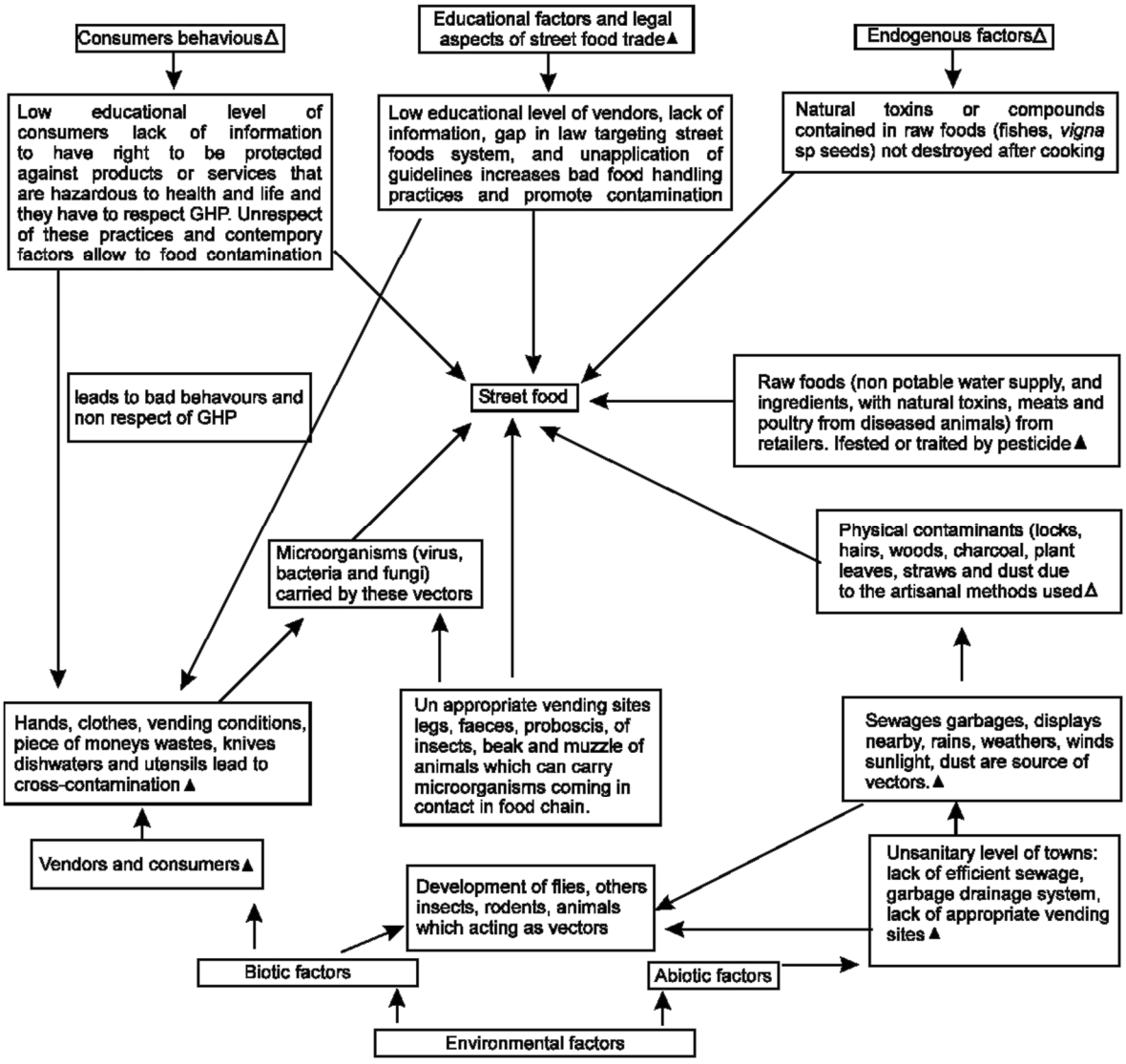

Fig 2.1 Mechanism of street-vended foods microbiological and physical contamination vectors and their routes diagram. (▲) major factors and (Δ) minor factors of contamination

2.3.3 Microorganisms associated with food

The microorganisms in food products do not arise by spontaneous generation; they must contaminate 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 equipments, ingredients, product to product, and packaging materials. Microorganisms can be exchanged between these sources. Rodents and flies play an important role in the transmission of disease-causing organisms.

The microbial flora of a food consists of the microorganisms associated with the raw material, those acquired during handling and processing, and those surviving any preservation treatment and storage. Microorganisms may have at least one of four functions in food. They may have useful function, cause spoilage, be a health hazard, or be inert. Microorganisms causing foodborne illness are of more concern than any other types.

The microbial analyses of food products yield many diverse types of microorganisms. Microorganisms isolated from red meat include genera of *Staphylococcus, Brevibacterium, Micrococcus, Bacillus, Lactobacillus, Corynebacterium, Flavobacterium, Pseudomonas, Alcaligenes, Streptococcus, Pediococcus, Acinetobacter, Microbacterium, Aerococcus, Salmonella, Clostridium, Vibrio* and 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, Fusarium, Penicillium, Phoma 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.* Yeasts and molds, especially spores, are also found in soil. The genera of bacteria that tend to be part of the normal flora of water include *Pseudomonas, Corynebacterium, Flavobacterium, Cytophaga, Acinetobacter, Moraxella, Flavobacterium, Aeromonas, Streptococcus, Klebsiella, Alcaligenes, Micrococcus, Bacillus* and *Escherichia*. The types of organisms in air are often associated with the type of activity in the area. Air is the major contributor of chemical pollutants (hydrocarbons, carbon monoxide, soot, fly ash) and biological agents such as plant cells, animal hair, pollen, algae, protozoa, bacteria, yeasts, mold spores, and viruses.

Humans are a source of airborne microorganisms as well as an important source of food contamination through handling of food. Human skin also contains relatively stable microflora. The skin is never free of bacteria, and the dirtier the skin, the greater the contamination. The bacteria found on the skin are *Staphylococcus, Corynebacterium,*

Micrococcus, Bacillus, Alcaligenes, Pseudomonas, Enterobacter, Klebsiella, Proteus, Escherichia and *Citrobacter*. *Staphylococcus aureus* is often found more often on the hands and face than other parts of the body. The organism is associated with the nose and is spread when the people handle their faces and noses.

A number of small equipments such as knives, cutting boards, bins, plates, etc. are used in food processing or handling establishments. Equipments may be cleaned and sanitized, but this does not mean that they are sterile. Even washed and visibly clean surfaces may have food deposits or films that provide microenvironments acceptable for survival and growth of microorganisms. During the course of daily operation, bacterial growth will occur in these food films and deposits. This then serves as a source of contamination when food contacts these surfaces. Similarly, the quality of processed food is influenced by the quality of the ingredients. Spices or seasonings are often the source of high microbial numbers, as high as 10^8 aerobic bacteria per gram. Also, they contain aerobic and anaerobic spores. Thermophillic bacteria, usually as spores, are added to foods with ingredients such as spices, starch, flour and sugar (Banwart, 2000b).

2.3.4 Microbial growth in food

Microbial growth usually refers to the reproduction-increase in population size, rather than to enlargement of cells. The average generation or doubling time in bacteria is 30-60 minutes under optimum conditions. Some species have very rapid growth rates and short generation time (5-10 minutes) and others have very slow growth rates and long generation time (measured in days). *Staphylococcus aureus* and *Salmonella enteriditis*, both causes of food poisoning, have relatively short generation time of 20-30 minutes, resulting in a few million cells from a few cells in few hours in the infested food (Aneja, 2003).

According to Kidiku (2001), microorganisms require food, moisture, warmth and time to grow and multiply. We cannot do much with the first two requirements, but warmth (temperature) and time we can control and/or manipulate and protect the food.

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 microorganisms. Foods 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).

Enumeration, isolation, detection, etc. are all very important routine activities in a microbiological laboratory. The microbiological testing of ready-to-eat foods should be appropriate to the type of food sample being examined and to the processing it has received. Not all the food borne organisms are equally applicable to all food groups, nor should all the organisms be routinely tested for. The significance of the microbiological tests that may be conducted is discussed below.

2.4.1 Indicator organisms

Routine examination of foods for a range of pathogenic microorganisms is impractical. In order to assess the microbiological safety from foodborne pathogens, widespread use of groups or species which are easily enumerated and whose presence in foods indicates exposure to conditions that might introduce hazardous organisms and/or allow their growth, are used. These groups are referred to as indicator organisms.

Indicator organisms are generally used to assess food hygiene; to indicate the presence of pathogens of intestinal origin as a result of direct or indirect faecal contamination. The main objective of using bacteria as indicators is to reveal conditions of treatment of the product which may imply a potential hazard that is not necessarily present in the specific sample examined, but could be present in parallel samples (Anon, 2009b).

The following indicator organisms have been used universally to determine the conditions foodstuffs are exposed to during handling.

2.4.1.1 Plate 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.

Most processed foods should be regarded as unwholesome when they have large populations of microorganisms, even if the organisms are not known to be pathogens. The reason is that high counts in shelf-stable foods often indicate contaminated raw materials in perishable products and may also indicate unsuitable time/temperature storage conditions. Some strains of common mesophilic bacteria, which are not commonly associated with foodborne disease, have been reported to cause illness when in excessive numbers. All recognized foodborne pathogens that are mesophilic will contribute to the detected plate count (Anon, 2009b).

Miskmin *et al*. (1976) suggested that the aerobic plate count is the most suitable method for evaluating the microbial quality of food and that where food safety is of concern, a search for specific pathogens should be made. An examination of the microbiological quality of a food should not be based on plate count alone.

2.4.1.2 Coliforms

The presence of enteric bacteria, e.g., coliforms in general and *Escherichia coli* in particular have been widely accepted as indicators of faecal contamination (not in the sense of implying immediate contact with the faeces) 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. 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 10^2 cfu/g indicates dangerous contamination of food.

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

a. Inadequate processing and/or post process recontamination due to cross-contamination by raw materials, dirty equipment or poor hygienic handling;

b. Microbial proliferation, which could have allowed multiplication of a wide range of pathogenic and toxigenic organisms (Anon, 2009b).

2.4.1.3 Yeasts and molds

In non-acid, moist foods, yeasts and molds grow more slowly than bacteria and therefore seldom cause problems in such foods. However, in acid foods and foods of low water activity, they outgrow bacteria and thus cause spoilage losses especially if the products (e.g., fresh fruit and vegetables, frozen or dried foods) are improperly stored. Additionally, there is also the potential hazard form production of mycotoxins by molds. Humans should not consume foods that are visibly moldy.

The presence of yeasts and molds is of little significance in fresh and frozen foods. One can expect to find small numbers of spores and yeast cells present in these foodstuffs. Consumers will recognize spoilage when very high numbers of yeast or visible moulds are present (Anon, 2009b).

2.4.1.4 Other indicator organisms

A number of other indicator organisms are also frequently used in assessing food safety, which includes Staphylococcal and mesophilic spore formers (Anon, 2009b).

a) The presence of Staphylococci is usually indicative of contamination from the skin, mouth or nose of food handlers. Inadequately cleaned equipment or raw animal products may also be sources of contamination. The presence of large numbers is in general a good indication of poor hygiene and temperature control. The presence of high numbers in cured meat may indicate the presence of enterotoxin producing strains of *S. aureus*.

b) The presence of mesophilic spore-forming bacteria in heat processed foods indicates that the heat processing is insufficient. When spore-forming bacteria are present in chilled or dried food in usually high numbers, there is a risk that they may include *C. perfringens, C. botulinum* or *B. cereus* which could present a hazard either in the foods as processed or in its future use.

2.4.2 Foodborne pathogens

Foodborne diseases can have a variety of causes, e.g., overeating; allergies; nutritional deficiencies; actual poisoning by chemicals; toxic plants or animals; toxins produced by bacteria; infestation by animal parasites; and infection by microorganisms. The term “food poisoning” is applied to diseases caused by microorganisms, which include both 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. *Escherichia coli, Salmonella, Campylobacter jejuni, 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).

2.4.2.1 *Salmonella*

With a few important exceptions, e.g. *Senterica* subsp. *typhi, S. dublin* and *S. choleraesuis,* salmonellae show little host specificity and most can cause gastroenteritis when ingested by humans. *Salmonella typhi* and *S. paratyphi* A, B and C are worthy of special mention. These serotypes are host adapted to humans, but can be transmitted in food. The usual source of these organisms in food is by contamination from an infected food worker or by direct contamination from human sewage (Roberts and Greenwood, 2003).

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

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).

2.4.2.2 *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 20^{o} and 50^{o}C, with maximum growth between 37^{o} and 47^{o}C. 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. Normally large numbers of cells have to be ingested to cause illness. Counts of 105/g are highly suggestive of the possibility of food poisoning occurring. Foods commonly associated with *C. perfringens* contamination include dairy products, pasta, flour, poultry and vegetables, which have been exposed to soil, dust and faecal material (Banwart, 2000c).

2.4.2.3 *Escherichia coli*

E. coli is an important organism in the microbiology of foods. An important reservoir of this organism is the intestinal tract of human, cattle and other food 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. The infectious dose is low (as low as 10^{1} - 10^{2}/g). Low doses cause illness in young children, the elderly and immuno-compromised persons. Foods implicated include undercooked hamburger patties and other fast foods and cheese made from unpasteurized milk (Anon, 2009b).

2.4.2.4 *Listeria monocytogens*

The organism is a small gram-positive rod with a tendency towards a diplobacillary form. Although, it is aerobic, it grows better at reduced O_2 and increased CO_2 levels. It is ubiquitous in nature (i.e. widespread in soil, food-processing environments, raw meats and faeces 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 $>10^{2}$/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, fermented sausages and coleslaw and other salads (Anon, 2009b).

2.4.2.5 *Shigella* spp.

The genus *Shigella* includes four species: *S. dysenteriae, S. flexneri, S. boydii,* and *S. sonnei*. These are not the natural inhabitants of the environment. The normal habitat is the

intestinal tract of human beings and other primates. Isolation from other animals is rare. These are host adapted organisms and only infect humans and other primates. Foods, which serve as vehicles include milk, vegetable salads, orange juice and cooked rice. The infective dose is small (10^1 - 10^2/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).

2.4.2.6 *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 >10^7/g normally cause disease in healthy persons. *V. parahaemolyticus* and *V. cholerae* are typically present in sea foods at levels below 10^3/g. Foods implicated in outbreaks are usually raw oysters and other molluscan shellfish (Anon, 2009b).

2.4.2.7 *Yersinia enterocolitica*

Yersinia enterocolitica is a member of the family Enterobacteriaceae. *Y. enterocolitica* can be found almost everywhere in nature, but only certain serotypes are involved in human infections. Pigs are believed to be the principal reservoir of serotypes pathogenic to man. It is sensitive to heat while resistant to other adverse storage conditions. The ability of the organism to grow at refrigeration temperatures makes refrigerated animal foods, such as milk, a potential hazard. The minimum infectious dose is uncertain. Foods implicated in outbreaks include unpasteurized milk, chocolate milk and raw pork (Anon, 2009b).

2.4.2.8 *Brucella* spp.

Species of importance include *B. melitensis*, *B. suis* and *B. abortus*. These can be isolated from animals such as cattle, sheep and goats. Brucellosis has become a disease of people who handle cattle and swine. Transmission to man can occur through contact with infected animals in the farm and during slaughter and through consumption of raw or unheated processed products of animal origin. Main vehicles of alimentary infections are raw milk and raw milk products, such as cream, butter and cheese. In milk, freezing supports survival, especially when contamination levels are high. These are unlikely to multiply in food (Anon, 2009b).

2.4.2.9 *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 –20^{o}C, but at higher temperatures ranging from –10^{o}C to 0^{o}C 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).

2.4.2.10 *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, 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 10^3/g or less, but mostly at levels less than 10^2/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 $>10^5$/g (Banwart, 2000c).

2.4.2.11 *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. It may occur on or in almost all foods whether of vegetative or animal origin. Although *C. botulinum* is widely distributed in the soil and on raw agricultural products, levels of contamination are generally low. Since huge quantities of agricultural produce are stored, processed in many ways and consumed by man, the possible survival of *C. botulinum* spores and their potential for growth and toxin production should be taken fully into account. Human botulism is commonly a result of eating improperly preserved foods (Anon, 2009b).

2.5 Microbial hazards in drinking water

Water used for cooking, drinking, dishwashing, etc. come from pipes or hand-operated tube wells. There is no certainty that the water of all the stalls within the same area is from the same source. The vendors store water in buckets or drums of galvanized iron which are refilled as needed. Drinking-water safety depends upon preventive approaches from catchment to tap. The greatest microbial risks are associated with contamination of drinking-water with human and animal excreta, although other hazards may also be significant.

Infectious diseases caused by pathogenic bacteria, viruses and protozoa or by parasites are the most common and widespread health risk associated with drinking-water. The pathogens that may be transmitted through contaminated drinking-water are diverse. For pathogens transmitted by the faecal-oral route, drinking-water is only one vehicle of transmission. Contamination of food, hands, utensils and clothing can also play a role, particularly when domestic sanitation and hygiene is poor.

Microbial water quality may vary rapidly and over a wide range. Short-term peaks in pathogen occurrence may increase disease risks considerably and may also trigger outbreaks of waterborne disease that may affect large numbers of persons. For these reasons reliance cannot be placed on water quality measurements alone, even when made frequently, to determine the safety of drinking-water.

Some of the pathogens that are known to be transmitted through contaminated drinking-water lead to severe and sometimes life-threatening disease. Examples include typhoid, cholera, infectious hepatitis caused by hepatitis A virus (HAV) or hepatitis E virus (HEV), and disease caused by *Shigella* spp and *E. coli* O157. Others are typically associated with

less severe outcomes, such as self-limiting diarrhoeal disease (examples rotavirus, *Cryptosporidium*). Whilst the latter are of limited importance to healthy adults, diarrhoeal disease is associated with significant infant morbidity and mortality in some regions and amongst immuno-compromised. More widely it contributes to malnutrition and thereby to developmental problems. Other health outcomes may be more significant amongst other age groups (WHO, 2003).

2.6 Categories of microbiological quality

Four categories of microbiological quality have been assigned based on standard plate counts, levels of indicator organisms and the number or presence of pathogens (Anon, 2001). These are satisfactory, marginal, unsatisfactory and potentially hazardous (Appendix B).

a. Satisfactory: Satisfactory results indicate good microbiological quality.

b. Marginal: Marginal results are border-line in that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the preparation of the food.

c. Unsatisfactory: Unsatisfactory results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices.

d. Potentially Hazardous: The levels in this range may cause food borne illness and immediate remedial action should be initiated.

2.7 Microbiological criteria for foods

A microbiological criterion for food defines the acceptability of a product or a food lot based on the absence or presence or number of micro-organisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot.

Microbiological criteria may be used to formulate design requirements and to indicate the required microbiological status of raw materials, ingredients and end products at any stage of the food chain as appropriate. They may be relevant to the examination of foods, including raw materials and ingredients, of unknown or uncertain origin or when other means of verifying that good hygienic practices are not available. Generally, microbiological criteria may be applied to define the distinction between acceptable and unacceptable raw materials, ingredients, products and lots by regulatory authorities and/or

food business operators. Microbiological criteria may also be used to determine that the processes are consistent with the recommended international code of practice (CAC, 2009).

The stated chief purposes of microbiological criteria for foods are to give assurance (1) that the foods will be acceptable from the public health standpoint, i.e. will not be responsible for the spread of infectious disease or for food poisoning, (2) that the foods will be of satisfactory quality, i.e. will consist of good original food materials that have not deteriorated or become unduly contaminated during processing, packaging, storage, handling, or marketing, (3) that the foods will be acceptable from an esthetic viewpoint in that the introduction of filth in the form of fecal material, parts of vermin, pus cells, mold mycelium, etc., has been prevented, and (4) that foods will have keeping qualities that should be expected of the product.

Standards must be adapted to the types of food for which they are intended. They probably would be different for a food to be consumed raw than for the same food to be cooked or subjected to heating or other processing before being marketed. The types of spoilage organism to be feared and therefore watched for vary with the food and the method of processing. The type of pathogens most likely to be present will be different in different foods (Fraizer and Westhoff, 2008f).

To evaluate the microbiological conditions of foods, the criteria that have been used are guidelines, recommended limits, specifications, and standards (Banwart, 2000d).

2.7.1 Guidelines

Microbiological guidelines have been developed for many foods. A guideline is used when no official standard exists for a particular food. Guidelines may be considered as administrative standards.

A guideline usually is an estimate of the microbial load that can be attained readily in a satisfactory plant using satisfactory methods of processing and materials.

Although not an official standard, a guideline can be useful for quality control personnel to make judgments about the acceptability of raw materials, finished products, or operations during processing.

2.7.2 Recommended limits

These are the suggested maximum acceptable numbers of microorganisms or of specific types of microorganisms, as determined by prescribed methods in a food. They are similar to guidelines.

There are many recommended microbiological limits. Most of these recommendations are based on too few samples or other considerations to be of much value. Due to the wide variation, it is evident that most of these are not valid.

2.7.3 Specifications

A specification is a document that provides a detailed description of a material. Included in a specification of a food may be acceptable microbiological quality. A microbiological specification has been defined as the maximum acceptable number of microorganisms or of specific types of microorganisms, as determined by prescribed methods, in a food being purchased by a firm or agency for its own use.

A practical specification should have tolerance limits set wide enough to allow permissible deviations. However, the limits should be narrow enough to ensure that the material will be acceptable consistently. The specification should include sampling and analytical procedures so that there is no question of the system that is to be used.

2.7.4 Standards

Above all the criteria, only microbiological standards have a regulatory function. It is that part of the law or administrative regulation designating the maximum acceptable number of microorganisms or of specific types of microorganisms, as determined by prescribed methods, in a food product, packed, stored, or imported into the area of jurisdiction of an enforcement agency.

A microbiological standard should not be established haphazardly. A standard must be meaningful and attainable by what is considered to be good manufacturing practice. Microbiological standards, per se, do nothing to increase the safety or quality of the food. It is the acceptance of the standards by all segments of industry and the enforcement by the regulators that is necessary before a standard has value.

2.8 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).

a. 74% of countries reported street-vended foods to be a significant part of the urban food supply;

b. Street-vended foods included foods as diverse as meat, fish, fruits, vegetables, grains, cereals, frozen produce and beverages;

c. Types of preparation included foods without any preparation (65%), ready-to-eat food (97%) and food cooked on site (82%);

d. Vending facilities varied from mobile carts to fixed stalls and food centers;

e. Infrastructure developments were relatively limited with restricted access to potable water (47%), toilets (15%), refrigeration (43%) and washing and waste disposal facilities;

f. 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

g. 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.

2.9 Strategies to enhance the safety of street vended foods

Strategies for improving street food should only be developed after appropriate studies and other information in local foods, conditions and practices have been obtained. The factors on which such strategies should be based are identified by preliminary studies of street food system and the HACCP based studies (WHO, 1996).

a. Preliminary studies of the street food system.

b. Policy, regulation, registration and licenses.

c. Location, infrastructure, services and design and concentration of vending units.

d. Training of food handlers.

e. Education of consumers.

f. Reinforcement of food safety measures for high risk occasion.

2.10 Food sanitation standards for street foods

For the operation of street food vendors, 12 practices or standard of operation must be followed. For restaurants this goes up to 15, and for cafeteria 30. The following are food sanitation standards for street foods (Kongchuntuk, 2002).

a. Food stall surface must be made from material that is easy to clean and must be in good conditions so as to permit easy and adequate cleaning. All food preparation or cooking area must be elevated to at least 60 cm above the ground.

b. Cooked food must be covered or stored in clean containers to prevent contamination from insects or pests.

c. Food additives used must be those that have been approved by authorized office.

d. Drinking water must be clean and fit for human consumption. It must be kept or stored in a clean and covered container with spout or draining valve.

e. Beverages must be stored in a clean and covered container with spout or draining valve. Long handle ladle may be used.

f. Ice must be clean and fit for human consumption. It must be kept in a clean and closed container that is elevated to at least 60 cm above ground. Long handle ladle is to be used to draw the ice and nothing shall be cooled or stored in this ice.

g. Food waste and trash must be collected and removed.

h. Wash utensils with dish washing detergent and rinse well under running water or twice in two water basin. This operation must also be elevated to at least 60 cm above ground.

i. Spoon, forks, chopsticks must be stored in open container with food circulation with handles up. These containers must also be elevated to at least 60 cm above ground.

j. Food handlers must wear clean cloth and the shirt must have sleeves. The cook must wear an apron and also a hat or hair net.

k. Always use clean utensils to pick up or serve food.

l. Any wound or cut on food handler's hand must be covered and protected with water-proof covering to prevent transmission of disease.

2.11 Challenges to food control activities

According to FAO/WHO (2005), a number of studies have revealed that food control activities, including control of street-vended foods, in under developed and developing countries have been hampered by a number of factors, including:

a. Inadequate or out of date food legislation,

b. Ill-equipped food inspectorates,

c. Inadequate laboratory facilities,

d. Poor management, and

e. Lack of coordination and cooperation among government food control agencies.

Part 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 July to September 2010. All the laboratory works were performed according to standard methodology as mentioned by Roberts and Greenwood in Practical Food Microbiology, 2003.

The reliability of the techniques for detection and enumeration of foodborne pathogenic micro-organisms has assumed critical importance. These techniques are (a) as simple and rapid as possible; (b) accurate and reproducible; and (c) economically feasible. Traditionally, microbiological analysis involves isolation, identification and confirmation of the desired micro-organisms (Speck, 1986).

3.1 Guidelines for microbiological analysis

An accurate microbiological assessment of the foods is possible only when the guidelines given below are followed:

a. Sampling: Numbers to be drawn.

b. Handling of samples: Exposure of samples to time and temperature during transit, i.e. before examination.

c. Sample preparation: Maceration and dilutions.

d. Enumeration procedure: Composition and preparation of plating media, plating methodology, incubation time-temperature and recording of the counts.

e. Confirmation and identification: Isolation of representative colonies, purification and determination of specific morphological, cultural and biochemical characteristics and need for confirmation of the organism to a specific group.

3.2 The study area

The research was carried out at Itahari from July to September, 2010. The study area was Itahari located in Sunsari district in the Koshi zone of south-eastern Nepal, where the climate was hot and the mean daily temperature was 26°C with a range of 18-37°C. The relative humidity was as high as 97% 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 plan

The Itahari municipality is divided into 9 wards. In total 8 sampling sites were chosen randomly with a purpose to obtain representative sample for study. Samples of *Momo, Chatpate, Panipuri, Alu chop, Pyaji* and *Dahibada* were collected aseptically from 8 different places in Itahari according to simple random sampling design. It is based on probability theory that every element of the population being sampled has an equal probability of being selected. At the same time, water sample was also taken from each place. And for each determination duplicate samples were taken.

Generally, the samples were collected in the afternoon (12-2 p.m.) based on their availability. During sampling, it was assumed that the quality of street foods collected do not change in short time (1-2 hrs), and if any, fairly regular. A sample size of about 100-250 gm, depending upon the type of sample, were collected in sterile polythene bags and analyzed within 2 hours of collection. The polythene bags were sterilized using 70% alcohol and the samples were placed inside them aseptically.

3.4 Chain of custody procedures

Properly designed and the executed chain of custody forms ensure sample integrity from sample collection to data reporting. This includes the ability to trace possession and handling of sample from the time of collection through analysis and final disposition. This process is referred to as “Chain of Custody” and is necessary to demonstrate sample control when data are to be used for routine control of samples.

The following procedures were performed in the present study:

a. Manual sampling was done.

b. Sample containers were made of polythene plastic packages, which were sterilized using 70% alcohol.

c. 100-250 gram samples were taken (based on sample type) to comply with the sampling, handling and analytical requirements.

d. Duplicate destructive samples were taken from each sampling sites.

e. Total sampling time took half an hour and the sample was transported in a protected condition to the laboratory within an hour of completion of sampling.

f. During analysis, parameters were processed with the prime priority and analyzed immediately.

3.5 Preparation of sample

3.5.1 Homogenization

Twenty-five gram of each samples 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. When blended properly, a 1:10 dilution of the food and associated microorganisms was obtained. This 1:10 dilution is also referred to as 1/10 or 10^{-1} dilution.

3.5.2 Serial dilution of homogenate

One ml of that sample homogenate (10^{-1} dilution) was pipetted and mixed in a 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^{-7} respectively as shown in Fig. 3.1 (KC and Rai, 2007).

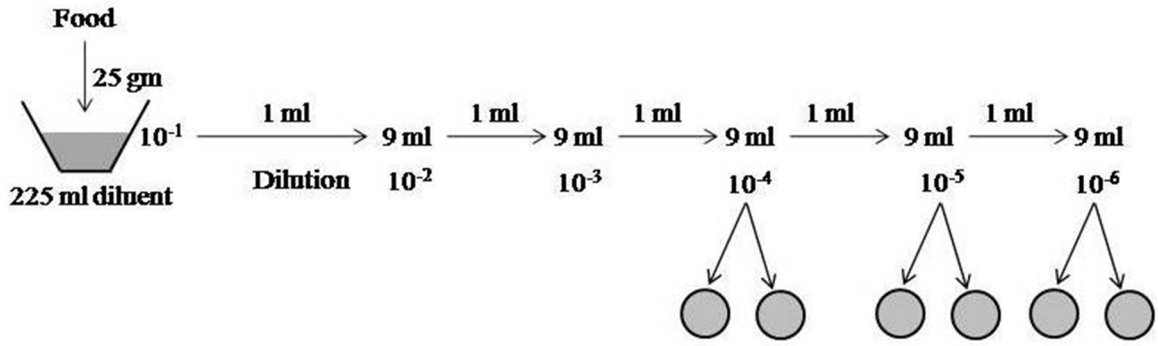

Fig. 3.1 Sample preparation for microbiological analysis

3.6 Media preparation

All the media including biochemical test reagents were prepared using the microbiology culture media manual (Anon, 2010c).

3.7 Plating and analysis

One ml aliquot of various dilutions prepared were transferred to sterile petri dishes (duplicate for each dilution) to which were added about 15 ml of sterile, liquefied (45^{o}C) agar medium. After each addition of agar, gently shaking (to and fro, or in the shape of 8) was done to ensure homogenous distribution of the organisms. Upon solidification, the plates were incubated in an inverted position. For total plate counts and total coliform counts, the plates with the number of colonies between 30 and 300 were selected and result was expressed as colony forming units per gram (cfu/g) using the formula:

$$\text{cfu/g} = \frac{\text{Number of colonies (average of two duplicates) x Dilution factor}}{\text{Dry weight of sample}}$$

3.7.1 Total plate count (TPC)

Total plate counts of food as well as water samples were determined by pour plate method according to Aneja (2003) using plate count agar media and distilled water as diluent.

3.7.2 Total coliforms

Total coliform counts of food and water samples were determined by pour plate method according to Roberts and Greenwood (2003) using violet red bile agar (VRBA) media.

3.7.3 Salmonella

Salmonella was detected according to Varadaraj (1993) with some modifications. The different steps involved are schematically represented in Fig. 3.2.

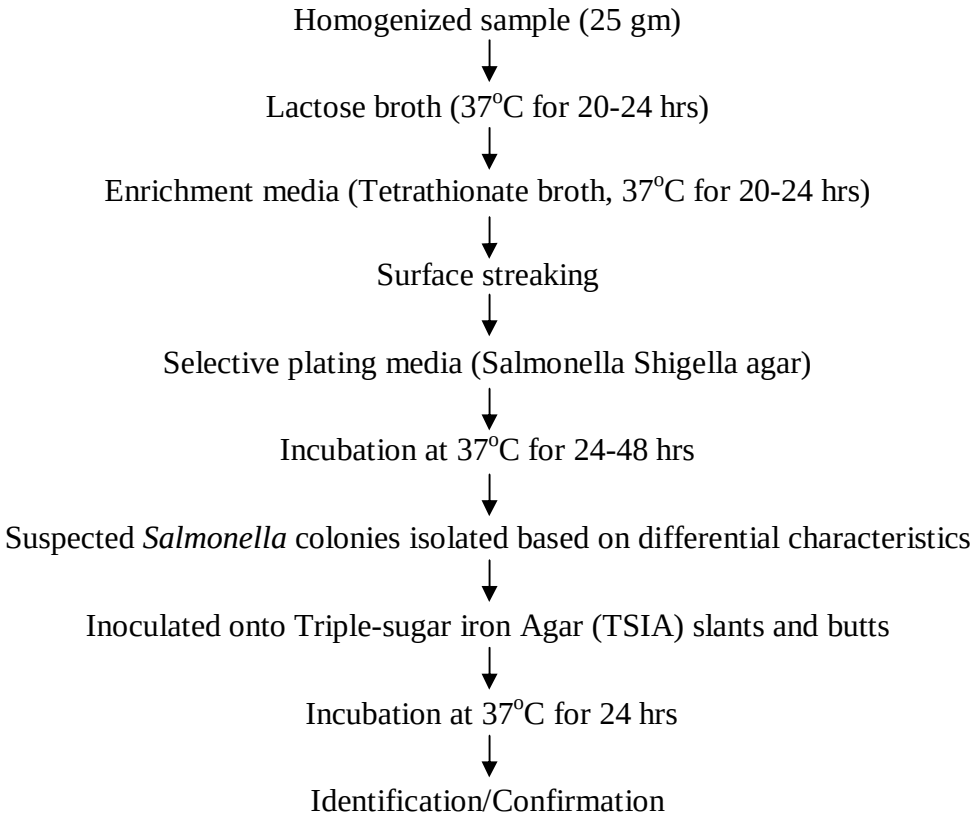

Fig. 3.2 Flow chart for the detection of *Salmonella* species

3.8 Confirmatory biochemical tests

Triple sugar iron agar (TSIA) medium was used for the preliminary identification of enteric pathogens. It is composed of three sugars: lactose (10%), sucrose (10%) and very small amount of glucose (1%), iron (ferrous sulphate), and phenol red as an indicator. The indicator was employed for the detection of fermentation of sugars indicated by the change in colour of the medium due to the production of organic acid and hydrogen sulphide.

In this process, if an organism ferments any of the three sugars or any combination of them, the medium will become yellow due to the production of acid as end product of fermentation. The entire pathogens, however, of fermenting only glucose and medium turns yellow within 24 hours of incubation, and in the aerobic conditions of the slant, the reaction reverts and becomes alkaline showing again the red colour in the slanted portion of the tube while the anaerobic butt will remain yellow (presence of acid) because the same organism is unable to cause a reversion in the anaerobic condition present in the butt. Thus *Salmonella* and *Shigella* show a yellow butt and a red slant, after 24-48 hours of incubation, indicating glucose fermentation only. No change in the medium indicates none of the sugars have been fermented (Aneja, 2003).

Production of gas from the fermentation of a sugar by an organism was indicated by the appearance of bubbles or splitting in the butt or pushing up of the entire slant from the bottom of the tube. Hydrogen sulphide (H_2S) production by any organism was indicated by the reduction of ferrous sulphate of the medium to ferrous sulphide, which is manifested as a black precipitate.

The procedure for the preparation of TSIA slants, biochemical reactions and scheme for differentiation of micro-organisms on the basis of the biochemical reactions are shown in Fig. 3.3, Fig. A.1 and Fig. A.2 (Appendix A) respectively.

Number the selected uncoloured different appearing colonies under the bottom of the media plate

↓

Label TSIA slants with numbers corresponding to those on the media plate

↓

Pick a few cells from the center of each colourless, transparent colony using a sterile inoculating needle

↓

Inoculate the organisms clinging to the tip of the inoculating needle to a TSIA slant by first streaking the surface of the slant and then stabbing the medium in the butt region

↓

Incubate the inoculated TSIA tubes at 35^{o}C for 24 hours

↓

Observe the tubes showing an alkaline slant and fermentation in butt, i.e. production of acid or acid and gas with or without hydrogen sulphide

Fig. 3.3 Flow chart for the preparation of TSIA slants

3.9 Data analysis

All the parameters analyzed except for *Salmonella* were statistically analyzed. The raw data were statistically processed for significant differences by ANOVA (Two factor without replication at 5% level of significance) in the computer using statistical Genstat program (Genstat discovery edition 3) provided by Lawes Agricultural Trust, and Data Analysis feature of Microsoft Excel (2007).

Part IV

Results and Discussion

4.1 Analysis of street food samples

Any food to be of good quality and safe for public health should be free from hazardous microorganisms. So in order to fulfill this requirement in this study, the total plate count and total coliform count were performed along with the detection of target pathogen like *Salmonella* spp. from six different samples (viz. buff *momo*, *chatpate*, *panipuri*, *aluchop*, *pyaji* and *dahibada*) collected from eight different places of Itahari municipality. The results of microbiological analysis of different food samples are presented in Table 4.1.

Table 4.1 Results of microbiological analysis of collected street food samples

S.N.	Place	TPC (cfu/g)	Coliforms (cfu/g)	*Salmonella*
Momo				
1	Bhetghat chowk	$1.7x10^2$	$0.3x10^1$	-
2	Biratnagar line	$1.9x10^2$	$0.4x10^1$	-
3	Bus park	$3.6x10^2$	$1.7x10^1$	-
4	Dharan road	$1.6x10^2$	$0.6x10^1$	-
5	Hatiya	$4.8x10^2$	$3.4x10^1$	-
6	Itahari chowk	$3.2x10^2$	$1.4x10^1$	-
7	Paschim line	$3.3x10^2$	$3.2x10^1$	-
8	Tyangra pool	$3.9x10^2$	$2.1x10^1$	-
C*hatpate*				
1	Bhetghat chowk	$9.4x10^4$	$1.4x10^3$	-
2	Biratnagar line	$7.8x10^5$	$8.2x10^3$	-
3	Bus park	$4.8x10^5$	$6.1x10^3$	-
4	Dharan road	$3.4x10^5$	$5.0x10^3$	-
5	Hatiya	$1.2x10^6$	$3.6x10^4$	-
6	Itahari chowk	$6.6x10^5$	$7.0x10^3$	-
7	Paschim line	$5.7x10^5$	$6.3x10^3$	-
8	Tyangra pool	$8.5x10^5$	$2.1x10^4$	-

Panipuri

1	Bhetghat chowk	$4.6x10^4$	$4.8x10^3$	-
2	Biratnagar line	$6.4x10^4$	$6.6x10^3$	-
3	Bus park	$7.8x10^4$	$7.2x10^3$	-
4	Dharan road	$6.2x10^4$	$5.9x10^3$	-
5	Hatiya	$3.9x10^5$	$1.4x10^4$	-
6	Itahari chowk	$5.7x10^4$	$6.5x10^3$	-
7	Paschim line	$3.4x10^5$	$8.1x10^3$	-
8	Tyangra pool	$3.5x10^5$	$1.2x10^4$	-

Aluchop

1	Bhetghat chowk	$9.7x10^2$	$2.6x10^2$	-
2	Biratnagar line	$8.9x10^2$	$2.2x10^2$	-
3	Bus park	$4.6x10^3$	$3.5x10^2$	-
4	Dharan road	$7.6x10^2$	$1.9x10^2$	-
5	Hatiya	$1.8x10^4$	$7.6x10^2$	-
6	Itahari chowk	$3.2x10^3$	$4.1x10^2$	-
7	Paschim line	$3.8x10^3$	$4.4x10^2$	-
8	Tyangra pool	$4.7x10^3$	$3.9x10^2$	-

Pyaji

1	Bhetghat chowk	$9.8x10^2$	$3.2x10^2$	-
2	Biratnagar line	$1.7x10^3$	$4.1x10^2$	-
3	Bus park	$1.6x10^4$	$2.1x10^3$	-
4	Dharan road	$9.3x10^2$	$2.6x10^2$	-
5	Hatiya	$2.3x10^4$	$1.9x10^3$	-
6	Itahari chowk	$4.4x10^3$	$5.3x10^2$	-
7	Paschim line	$3.8x10^3$	$5.0x10^2$	-
8	Tyangra pool	$8.7x10^3$	$1.2x10^3$	-

Dahibada

1	Bhetghat chowk	$3.4x10^5$	$4.7x10^3$	-
2	Biratnagar line	$5.1x10^5$	$6.5x10^3$	-
3	Bus park	$8.0x10^5$	$8.8x10^3$	-
4	Dharan road	$4.0x10^5$	$5.4x10^3$	-
5	Hatiya	$8.7x10^5$	$1.6x10^4$	-
6	Itahari chowk	$7.2x10^5$	$8.3x10^3$	-
7	Paschim line	$7.6x10^5$	$1.1x10^4$	-
8	Tyangra pool	$8.1x10^5$	$1.2x10^4$	-
	Avg (*Momo*)	$3.00x10^2$	$1.64x10^1$	
	Avg (*Chatpate*)	$6.22x10^5$	$1.14x10^4$	
	Avg (*Panipuri*)	$1.73x10^5$	$8.14x10^3$	
	Avg (*Aluchop*)	$4.62x10^3$	$3.78x10^2$	
	Avg (*Pyaji*)	$7.44x10^3$	$9.03x10^2$	
	Avg (*Dahibada*)	$6.51x10^5$	$9.08x10^3$	

(Note: TPC = Total plate count, cfu/g = Colony forming units per gram, - = negative. Avg = Average values of microbial load of sample mentioned in parenthesis)

Many varieties of street foods are being sold in Itahari market, all of which may not assure in terms of microbial quality. Microorganisms set into the food products by water, unclean utensils, knives, unscientific processing practices and cruel handling methods, besides, environmental contamination, handling in their preparation and sales, lack of scientific methods of storage and due to lack of knowledge of microorganisms. Once microorganisms are introduced into the food products, they multiply rapidly and reach levels sufficient to produce infections or intoxications depending upon the types of invasion. The increasing population, urbanization, and modernization of the Itahari city are also responsible for the pollution, the impact of which is on various food borne diseases due to the contamination by various pathogenic bacteria.

The number of microorganisms in any food sample 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 or 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. Even though the microbial population in the street foods does not cause food borne disease, certain microbial contamination is an indicator of poor sanitary practice in their processing and storage. Moreover, the eating habits and tastes of people have also come to vary which has further influenced the emergence of numerous food stalls. Hence, there is an urgent need to know the quality of food that is provided in the food establishments.

Microbial examination of final product does not reveal information of the point of contamination nor ensures protection against. However, it gives the idea of hazard quality. Table 4.1 shows the total plate count, total coliform count and presence/absence of *Salmonella* in six different street food samples collected from eight different places. A total of 48 street food samples were analysed. The total plate counts of all the samples were in the range of $1.6x10^2$ to $4.8x10^2$, while the total coliform counts were in the range of $0.3x10^1$ to $3.6x10^4$. *Salmonella* was not detected in any of the samples.

The average TPC were found to be $3.00x10^2$, $6.22x10^5$, $1.73x10^5$, $4.62x10^3$, $7.44x10^3$ and $6.51x10^5$ cfu/g in *momo*, *chatpate*, *panipuri*, *aluchop*, *pyaji* and *dahibada* samples respectively. Similarly, the total coliform counts were $1.64x10^1$, $1.14x10^4$, $8.14x10^3$, $3.78x10^2$, $9.03x10^2$ and $9.08x10^3$ cfu/g in *momo*, *chatpate*, *panipuri*, *aluchop*, *pyaji* and *dahibada* samples respectively. Above figures suggested that the microbiological quality was not satisfactory. The total plate counts and total coliform counts were higher in all samples indicating poor sanitary condition during processing. Data analysis (Appendix D) showed that the microbial load were found to be significantly different within the samples and sample types collected from different locations for Table 4.1 at $p \leq 0.05$. This indicated that the hygienic practice (keeping other things constant) in the street food stalls were significantly different.

Karki (2005) reported that the average total plate counts were found to be 4.31×10^4, 8.38×10^4, 2.62×10^2 and 1.35×10^4 cfu/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 1.70×10^4, 6.77×10^3 and 1.35×10^4 cfu/g in *chatpate*, *dahibada* and *panipuri* samples respectively. Similarly, Dahal (1993) reported that the total plate counts in *chatpate* and *dahibada* samples collected from Dharan markets were in the range of 5.7×10^6 to 1.1×10^8 and 1.8×10^7 to 1.2×10^8 cfu/g respectively, whereas coliform

counts in the same were in the range of 1.0×10^4 to 9.3×10^4 and 1.0×10^4 to 9.0×10^4 cfu/g respectively (Appendix F). It shows that the hygiene of street foods is very poor in Nepal as a whole.

The total plate count was found to be higher in *momo, chatpate, panipuri* and *dahibada* than reported Karki (2005). On the other hand, 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 *momo* samples was found to be satisfactory, whereas the same for the remaining samples analysed 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.

A total of 48 street food samples were analysed. Based on ICMSF guidelines, among the foods analysed, 8.3% had unsatisfactory total plate count, while 89.6% had unsatisfactory total coliform count (Table 4.2).

Table 4.2 Microbiological results of food according to parameter of analysis

Parameters of analysis	*Momo*		*Chatpate*		*Panipuri*		*Aluchop*		*Pyaji*		*Dahibada*		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
1. Total plate count														
Satisfactory	8	100	7	87	8	100	7	87	6	75	8	100	44	91.7
Unsatisfactory	0	0	1	13	0	0	1	13	2	25	0	0	4	8.3
2. Total coliform count														
Satisfactory	5	63	0	0	0	0	0	0	0	0	0	0	5	10.4
Unsatisfactory	3	37	8	100	8	100	8	100	8	100	8	100	43	89.6

Although most of the samples had satisfactory TPC, the range is high. The main sources of microorganisms are air, water, handler and container. Foods become contaminated during preparation and vending to the market. Most of the venders expose their products in the pushcart/stall openly, due to which microorganisms from air have greater chances of

invasion and proliferation. Coliform bacteria are pathogen to human. From analysis, it showed that most of the samples had unsatisfactory total coliform count. According to Eley (1992), the presence of high numbers of coliforms in foods indicate inadequate cleaning, unsanitary handling and post processing contamination from dirty atmosphere around shops/stalls. Parameters such as insufficient cooking, use of raw vegetables, cross-contaminations (between raw and cooked food, and contaminated ingredients), improper use of food container, unclean area of food preparation and inadequacy/improper garbage bin are significantly associated with unsatisfactory co1iform count.

Foods that we eat pass through different stages of processing and the sanitary condition of processing and storage places, personnel hygiene are of paramount importance so far as the food quality is concerned (Karki, 2005). The other factors influencing the number of microorganisms in any food include the raw materials used, nutritive value, pretreatments, and physical and chemical properties of foods (moisture content, water activity and possible presence of inhibitory substance).

According to Thapa *et al.* (2008), the time/temperature exposure of 66°C-72°C for 10 to 15 minutes for steaming is sufficient to reduce or kill the micro-organisms. Steaming/cooking for sufficient period of time-temperature can reduce the coliform organism to the levels independent of the quality of raw materials. If cooking is done for sufficient period of time-temperature, it fully eliminates the vegetative cells of harmful microbes. The final steamed product is safe for human consumption until and unless the serving plate and handling practices is hygienically good.

In the preparation of *momo* (buff), buffalo meat is the major ingredient. The quality of meat has significant influence on the quality of the product. Adhikari (2006) reported that the average total plate count and coliform count in buffalo meat samples collected from Dharan markets were $3.59x10^7$ and $2.06x10^4$ respectively. Similarly, Gautam (2007) reported the average total plate count and coliform count in buffalo meat samples collected from Itahari markets were $2.4x10^7$ and $2.1x10^4$ respectively. This indicates that the quality of buffalo meat used and insufficient steaming are cause of high microbial count.

The other reasons for the presence of TPC and coliform in significant number in *momo* could be due to post-preparation contamination. This relates to the sanitary condition, personal hygiene of the employees and the handling practices as well as the raw materials used for the preparation of *momo,* in case the steaming/cooking has not been carried out for

sufficient time-temperature combination. The same applies for *aluchop* and *pyaji*, as both are deep fried products in which the microbial counts, as such, should be low.

In the preparation of *chatpate*, raw vegetables are the major ingredients, which as such have high microbial count. Water is also one of the ingredients which help to increase the number of microorganisms in the sample. The serving material, i.e. paper, also contributes to high microbial count. Beside these, it does not undergo any heat processing/treatment as well. The same is true in the preparation of *dahibada*. In *dahibada*, dahi is one of the major ingredients and it is the excellent medium for the growth of microorganisms. The other ingredients such as spices, coriander leaves, etc. also increase the microbial count.

According to Das *et al.* (2010), bacterial enumeration of *panipuri* revealed a total viable count of $0.4x10^4$ to $3.0x10^4$ cfu/g. 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. This might also implicate the processing and rinsing water as possible sources of contamination of *panipuri* sold by street vendors (Khalil *et al.*, 1994).

Vendors-sold foods usually make use of simple facilities like wheel barrows, trays, mats, tables and make-shift stalls, thus further increasing the risk of food contamination. Contamination from raw materials and equipments, additional processing conditions, improper handling and prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens (Mahale *et al.*, 2008). The use of dirty utensils, as well as open display of street foods encourages visits by flies, cockroaches, rodents and dust. Preservation of prepared foods that requires no further processing before consumption, at ambient temperatures during retail, maintenance of the food at optimum temperatures, allow the invasion by pathogenic mesophiles (Bryan *et al.*, 1992).

The bacteriological quality of food indicates the amount of bacterial contaminants it has; a high level of contamination indicates low quality and more likely to transmit infection and the reverse is true. Presence of TPC and coliforms in significant number may be due to various reasons, including methods of food preparation, place of preparation of street foods, environmental surrounding of the street food vendors, handling of street foods, storage of prepared street foods before selling, cooking and serving utensils, personal hygiene of the vendors, and methods for packaging and storage of leftovers (Anon, 1988).

Many a times the street foods are sold by unlicensed vendors with poor education level and untrained in food hygiene. Most vendors acquire cooking skills from observation, some are taught by their parents while few gain the skills by trial and error (self taught). Most of the vendors neither undergo any form of formal training in food preparation nor do they attempt to seek it. Most of the vendors have had either no formal education or few years of schooling and therefore, lack knowledge on proper food handling and their role in the transmission of pathogens (Mensah *et al.*, 2002). According to FAO, food handlers should have the necessary knowledge and skills to enable them to handle food hygienically. FAO recommends that every vendor/helper of food should undergo a basic training in food hygiene (FAO, 1988).

Street food vendors prepare the foods either at home or at the stalls, which are located by the roadsides. Most stalls are poorly constructed and could not give proper protection of the foods from dust and smoke from vehicles. Handling of foods at ground level leads to the contamination because dust is easily blown on the food thus handled. Dust carries many microbes that may be pathogenic if left to settle on prepared foods. FAO recommends that foods should be prepared in a place set aside exclusively for that purpose, while the place of preparation should be kept clean at all times and should be far from any source of contamination (rubbish, waste water, dust and animals) (FAO, 1988).

Most of the vendors prepare their foods in unhygienic conditions, such that garbage and dirty waste are conspicuously close to the stalls. Also, most of the vendors do not have garbage receptacles; hence they dispose their garbage just near the stalls. They throw waste water just beside the stalls making the environment surrounding the eateries quite filthy. Houseflies are also present in most of the stalls. Presence of flies is an indication of poor hygiene and sanitary practices. Flies are manually controlled, no special attention are paid to control the flies (Muinde and Kuria, 2005).

Vendors do not wash fresh foods properly. Vendors wash their raw foodstuffs only once because they lack enough water. The preparation surfaces used by the vendors also contain remains of foods prepared earlier. More than one food types when prepared at the same surfaces could promote cross contamination. The oil used for deep-frying purpose is used more than once. The use of the recycled oil makes the deep fried products to have an unusual dark colour and unpleasant odor (Muinde and Kuria, 2005).

Vendors usually cook their food fresh every morning and sell it throughout the day. Cooked foods are kept in different ways (i.e. openly, in bowls) before they are sold. Most of the foods are not covered and exposed to flies and dust. Vendors store and serve prepared foods at ambient temperatures. Food is not heated at high temperatures before serving. Foods to be eaten raw like fruit salads are not kept under cold temperatures. Also, in busy places, the foods are not cooked properly; holding time-temperature is the effective factor which has direct effect on microbial load of foods (Muinde and Kuria, 2005).

Several authors report increase in mesophilic aerobic bacteria when the duration of holding was prolonged, indicating microbiological deterioration of food (Bryan *et al.*, 1997). Cooked foods are subjected to cross contamination from various sources (Fig 2.1). The outbreak itself, is always associated with the initial contamination of a ready-to-eat food such as street vended foods and re-contamination by inappropriate handling after cooking. Reheating is a "magic step" for eliminating hazards resulting from improper holding. Reheating at 75^{o}C for 15 seconds is an important action to ensure the food's safety (WHO, 2002).

Food service utensils used by the vendors are made from plastic, metal, enamel or are disposable polythene/paper containers. The paper used for serving food is usually newsprint of questionable origin. Most of the vendors wash their utensils in cold water, rinse only once and the water is used repeatedly before it is replaced. The water for washing and rinsing the utensils are observed to be dirty. Sometimes, venders and hawkers do not clean the container up to few days. The cleanliness of the utensils, knife and other contact surfaces are equally responsible for the poor hygiene quality of the street vended foods (Muinde and Kuria, 2005).

Vendors do not wear aprons or caps, and they handle foods with bare hands. Handling with bare hands may result in cross contamination, hence introduction of microbes on safe food. The person handling food also handles promissory notes and coins that are considered dirty and can cross-contaminate safe food. When packing foods in polythene bags, they blow air into the polythene bags to open them so that they could put the food in them. Clearly germs, some fairly harmful can become food-borne through this process. The lack of public sanitary facilities could be another hurdle to keep the desirable hygiene of the hands of the street food vendors. Personal hygiene is important because human beings are the largest contamination sources of food (Muinde and Kuria, 2005).

Most of the street food vendors usually have leftovers. Some consume them and the rest store them for the following day's sale. Proper methods of storing leftover food are not used; hence this could promote the sale of stale food. At an international conference on nutrition it was resolved that if food cannot be served immediately, it should be kept hot or cooled down rapidly and reheated completely to a temperature of at least 70^{o}C before eating. This is to make sure that microbes will not thrive on the food because there they flourish well between 10^{o}C and 60^{o}C. It is recommended that the street food vendors prepare enough food for the day, so that they can sell all the food since most of them do not have good storage facilities (WHO, 2002).

In India, street foods are sold at all public places and roadside shops. However, their consumption, quick method of cleaning and handling, could often prove to be a public health threat. There are reports of food borne illnesses associated with the consumption of unhygienic foods at several places in India. HACCP conducted for a selected *bhelpuri* vendor from urban Vadodara, that involved microbial analysis of 8 ingredients of *bhelpuri* and 7 samples indicative of personal hygiene and environmental sanitation showed the presence of *E. coli* in almost all the samples and *Salmonella* and *Shigella* in knife, hand rinse and dishwater samples (Sheth *et al.*, 2005). Bacteria like *Salmonella* spp. *Shigella* spp. *Campylobacter* spp. and *E. coli* can contaminate the food through contact with sewage and contaminated water (Beuchat, 1996). The hazards and critical control points identified were high initial contamination of raw foods, poor personal hygiene and environmental sanitation, cross-contamination between raw and cooked foods, holding of foods at ambient temperature and poor cleaning practices for stall and utensils (Sheth *et al.*, 2005).

In the present study, the environmental conditions under which street vendors worked were different from each other. The diversity of street foods is extensive, as they vary widely not only from country to country, but also from vendor to vendor. Street food ingredients are country specific and mostly undocumented. There are so many varieties that it is impossible to provide a menu of all the different street foods consumed around the world. The ingredients and means of preparation are equally diverse.

Information on street foods in developing countries is not readily available. However, studies on street-vended foods in USA, Asia, and a few African countries have revealed high bacterial counts and presence of foodborne bacterial pathogens. Total plate count exceeding 4×10^{5} cfu/g have been reported for vegetable salads and pepper sauce served

with street foods in many countries (Mosupuye & Von Holy, 1999; Bryan *et al.*, 1997). According to WHO (2002), effects of microbiological hazards such as *Salmonella* and *Escherichia coli* on food safety is now a major public health concern worldwide.

4.2 Analysis of water samples used by street food vendors

At the time of collecting street food samples, water was also collected from each location. Most of the street food vendors use the same water for cleaning purpose as well as for serving purpose. The water samples were analyzed for total plate count, total coliform count and *Salmonella*. The results of microbiological analysis of water samples used by street food vendors obtained from eight different locations of Itahari from where the street food samples were collected are presented in Table 4.3.

Table 4.3 Results of microbiological analysis of water samples

S.N.	Place	TPC (cfu/g)	Coliforms (cfu/g)	*Salmonella*
1	Bhetghat chowk	$8.4x10^1$	$2.2\ x10^1$	-
2	Biratnagar line	$1.3x10^2$	$3.5\ x10^1$	-
3	Bus park	$1.3x10^2$	$3.9\ x10^1$	-
4	Dharan road	$9.3x10^2$	$3.1\ x10^1$	-
5	Hatiya	$1.7x10^2$	$4.4\ x10^1$	-
6	Itahari chowk	$1.2x10^2$	$3.7x10^1$	-
7	Paschim line	$1.5x10^2$	$4.3\ x10^1$	-
8	Tyangra pool	$1.4x10^2$	$4.8\ x10^1$	-
	Avg	$1.28x10^2$	$3.74x10^1$	

(Note: TPC = Total plate count, cfu/g = Colony forming units per gram, - = negative. Avg = Average value of microbial load of water samples)

Drinking water is indispensable for human existence. It is essential that all living beings should get clean, pure drinking water. Simple access to safe water and adequate sanitation is an essential first step to protect human health, and a basic human right. Lack of access to safe drinking water and poor sanitation still threatens the health of millions of people.

Diseases caused by contaminated water are among the ten most prevalent water borne diseases in Nepal. Diarrhoea, which is caused by poor sanitation, hygiene and water quality, is one of the most prevalent water borne disease in Nepal. During 1995/96, the

incidence of diarrhoea among children below five years of age was 131 per 1,000 children. The mortality rate due to the diarrhoea was 0.34 per 1000 children under five years of age, while the case of fatality rate was 2.56 per 1,000 (CBS, 2001).

In Nepal, the incidence of diarrhoea is increasing in alarming rate. Historically, water has played a significant role in the transmission of human disease. The most important pathogenic bacteria transmitted by the water route are *Salmonella typhi*, the organism causing typhoid fever, and *Vibrio cholerae*, the organism causing cholera (Prasai *et al.*, 2007). The WHO (1993) has suggested that up to 80% of all human illness in developing world is caused by inadequate sanitation or polluted water. Diseases related to water, sanitation and hygiene risk factor kill more people than AIDS, malaria or tuberculosis (NHRC/WHO, 2002).

Ideally, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Detection of faecal indicator bacteria in drinking water provides a very sensitive method of quality assessment and it is not possible to examine water for every possible pathogen that might be present (WHO, 1993).

Of all the contaminants in drinking water, human and/or animal faeces, which may enter water routes through different means, present the greatest danger to the public health. Bacteriological testing provides a sensitive means for the detection and control of such pollution. Table 4.3 shows that the total plate count, total coliform count and presence/absence of *Salmonella* in eight different water samples collected from different places of Itahari municipality. The average total plate count and coliform count are $1.28x10^2$ and $3.74x10^1$ cfu/g respectively. *Salmonella* was not detected in any of the samples. The microbial load were found significantly different within the water samples collected from different locations for Table 4.3 at $p=0.05$ (Appendix E).

The main objective of this portion of study was the evaluation of quality of water used by street food vendors for drinking purpose. Total plate counts and total coliform counts have been used extensively as a basis for regulating the microbial quality of drinking water. In this study, both regulatory parameters were excessively above the WHO guideline values (Appendix C). Study results clearly indicated that most of the water sources are highly contaminated and the quality of the water consumed is critical in controlling infectious diseases and other health problems. The high microbial level can be assigned to poor sanitation habit due to the lack of hygiene education.

In Itahari city, the drinking water supply is regular. Also, nearly all of the surface sources and ground water sources have been exploited. Water quality indicates that pollution of the water is increasing alarmingly. The quality of the water supply is not safe mainly because of the bacteriological contamination. The situation of drinking water is degrading due to rapid population growth, unplanned urbanization, and poor sanitation of its infrastructure. There are several reasons behind the poor quality of water delivered to the households. Not all water distributions have appropriate treatment facilities. Either, water is improperly disinfected or not disinfected at all. Because of discontinuity of water supply and leakages, negative pressure often draws contaminated material from the surface. Even good quality of water delivered from the source gets polluted due to infiltration of contaminated water through the leakage points. The problem is worsened by the old distribution network. All the natural water sources, such as wells, rivers and tube wells are neither treated nor protected properly. The quality of surface water has deteriorated because of direct discharge of untreated sewage into rivers.

One of the other major sources of contamination of foods sold by street vendors is the washing and processing water (Khalil *et al.*, 1994). It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic condition related to improper washing of fruits, vegetables and utensils.

Local street vendors have limited access to clean running water. Water that is collected in the morning is frequently used until the end of the day. This result in vendors using little water for washing utensils and hence hygiene is compromised. Running water is not available. Without enough water, hygiene and sanitary practices cannot be met. Personal hygiene can only be achieved if adequate water is available. Therefore, vendors should have sufficient potable water for drinking, preparation of all kinds of foods and sufficient running water for all washing operations. Adequate drainage and waste disposal systems and facilities should be provided in the street food industry and designed properly so that the risk of contamination of food and potable water is low.

A regular monitoring the water quality for improvement not only prevents disease and hazards but also checks the water resources from going further polluted (Trivedy and Goel, 1986). The conservation of water sources is very important to provide safe water. As far as possible, water sources must be protected from contamination by human and animal waste, which can contain a variety of bacterial, viral, protozoan and helminthes parasites.

Part V

Conclusion and recommendations

5.1 Conclusion

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 Itahari 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. Randomly eight different locations were chosen to collect street food samples of six different varieties along with eight water samples and microbiological analysis were carried out to enumerate total plate count, total coliform count and *Salmonella*. Following conclusions can be drawn from the research work executed.

1. All the street food samples were found to contain higher microbial load than standards prescribed by ICMSF.
2. The total plate counts and total coliform counts were surprisingly higher in all samples indicating inadequate cleaning, unsanitary handling and post processing contamination due to poor sanitary condition of shops/stalls, handling and processing premises. Consumption of these foods may be harmful to human health.
3. None of the samples were found to contain *Salmonella*.
4. The microbial load were found to be significantly different within the samples and sample types collected from different locations indicating that the hygienic practice (keeping other things constant) in the street food stalls are significantly different.
5. Considering individual samples, the microbial load were higher in *chatpate, panipuri* and *dahibada* as compared to *momo, aluchop* and *pyaji*. The degree of contamination is dependent upon the hygienic condition of their localities and the raw material, way of handling and processing.
6. The quality of water used by street food vendors is also critical. Both of the regulatory parameters, viz. total plate count and total coliform count, were excessively above the WHO guideline values.

7. The microbial load were found to be significantly different within the water samples collected from different locations, indicating that the hygienic practice (keeping other things constant) in the street food stalls are significantly different.
8. The findings suggest about the unhygienic and unscientific method of handling, lack of sanitation and knowledge of microorganisms resulting higher degree of contamination.

The overall quality of street foods vended in the market of Itahari municipality was found to be quite unsatisfactory when compared with the standards given by ICMSF. When considered to the developing country like Nepal, where no microbiological standards are found regarding street foods and level of awareness, facility and infrastructure for the food processing premises are on the lower level, the profile of microbes could be considered as not safe and there is strict need of awareness campaign.

5.2 Recommendations

The microbiological study of the street foods vended in the market of Itahari municipality has been carried out in order to focus on their bacteriological profiles. All the samples were found to contain significant number of microorganisms, indicating the quality of street foods being very poor. There is need for concerted efforts to improve the safety of street-vended foods and the livelihood of street food vendors. Taking these considerations, following recommendations are given to the people, municipality and the government.

1. Food vendors should be encouraged to operate from designated places. Local authorities should provide the informal vendors with appropriate facilities where they can carry out their activities, including well-designed shelters/stalls, ample supply of potable water, sanitary facilities and waste disposals. All these should be done in consultation with the vendors in order to develop user-friendly sites.
2. Food legislation and guidelines should be developed to recognize the street food industry by developing code of practice for street food vending. Food laws should be adapted to changing circumstances retaining the ability to ensure the safety of the food. Food vendors should be aware of the provisions of the laws governing street foods.
3. FIFO (First-In-First-Out) approach should be applied for both quality and safety reasons. FIFO means that the first batch of the product prepared should be the first one sold. The FIFO concept limits the potential for pathogens growth, cross contamination, and encourages product rotation.

4. Every vendor, helper or food handler should be trained in all issues pertaining to their business such as hygiene, food laws and financial matters. Awareness campaigns should be carried out through the radio, television, posters and billboards.
5. Regular checks on quality of street foods by concerned authority need to be strictly implemented for public health protection.
6. Licensing of street food vendors should be done, which aids as communication channel between the municipal officials, health inspectors and food vendors.
7. Consumers should be informed of the requirements for healthy and safe food, especially street vended foods.
8. Aside the major recommendations of providing basic infrastructure and enforcement of bye-laws and codes of practice on street foods, the vendors should emphasize on the following simple steps in keeping food safe from harmful microorganisms:
    a. Vendors suffering from communicable disease should not be involved in food preparation or any sort of activities related to food.
    b. Properly washed good quality raw materials should be used.
    c. Good sanitary practice should be followed throughout the handling, preparation and serving of foods.
    d. Street food vendors should prepare enough food for the day, so that they can sell all the food without left-over.
    e. Food should be cooked adequately and re-use of leftover food should be avoided.
    f. Foods should be kept separately to prevent cross contamination between raw (or minimally-processed) and cooked foods.
    g. Reuse of the same cooking oil should be avoided.
    h. Water used for cleaning and washing should be of microbiologically safe quality and same water should not be used repeatedly.
    i. Use of unhealthy serving items like plates, cups, spoons made up of plastics and newspapers should be avoided.
    j. Proper waste/garbage management should be carried out.

Part VI

Summary

Six different samples of street foods, viz. *momo, chatpate, panipuri, alu chop, pyaji* and *dahibada* were collected from eight different locations of Itahari municipality for the enumeration of total plate count and total coliform count. *Salmonella* was also checked for their presence and absence. Also eight different samples of water used by street food vendors were collected to enumerate the same.

The average value for total plate count were found to be $3.00x10^2$, $6.22x10^5$, $1.73x10^5$, $4.62x10^3$, $7.44x10^3$ and $6.51x10^5$ cfu/g in *momo, chatpate, panipuri, aluchop, pyaji* and *dahibada* samples respectively. Similarly, the total coliform counts were $1.64x10^1$, $1.14x10^4$, $8.14x10^3$, $3.78x10^2$, $9.03x10^2$ and $9.08x10^3$ cfu/g in *momo, chatpate, panipuri, aluchop, pyaji* and *dahibada* samples respectively. The average value for total plate count and coliform count in water samples were found to be $1.28x10^2$ and $3.74x10^1$ cfu/g respectively. *Salmonella* was not detected in any of the samples.

Data analysis showed that the microbial load were found to be significantly different within the samples and sample types collected from different locations for all samples at p=0.05. From the result, it was observed that bacterial contamination of the street foods is dependent upon the micro-flora of possible contaminating sources and hygienic conditions of their localities, irrespective of their processing.

From all the findings, it was clear that the production and vending of the street foods were unhygienic. The methods of handling, processing and serving need to be upgraded. All the vendors should be well trained for the production of hygienic foods and healthy environment. Waste disposal places should be clearly allocated. Strict implementation of hygienic standards may help reduce contamination of street foods. Food safety regulation should be improved and fully implemented, through legislative measures wherever suitable and applicable, but with much greater reliance on voluntary compliance and education of consumers and professional food handlers. This must be the important task for the local authorities aiming at “health for all”.

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Appendices

Appendix A

Confirmatory biochemical tests

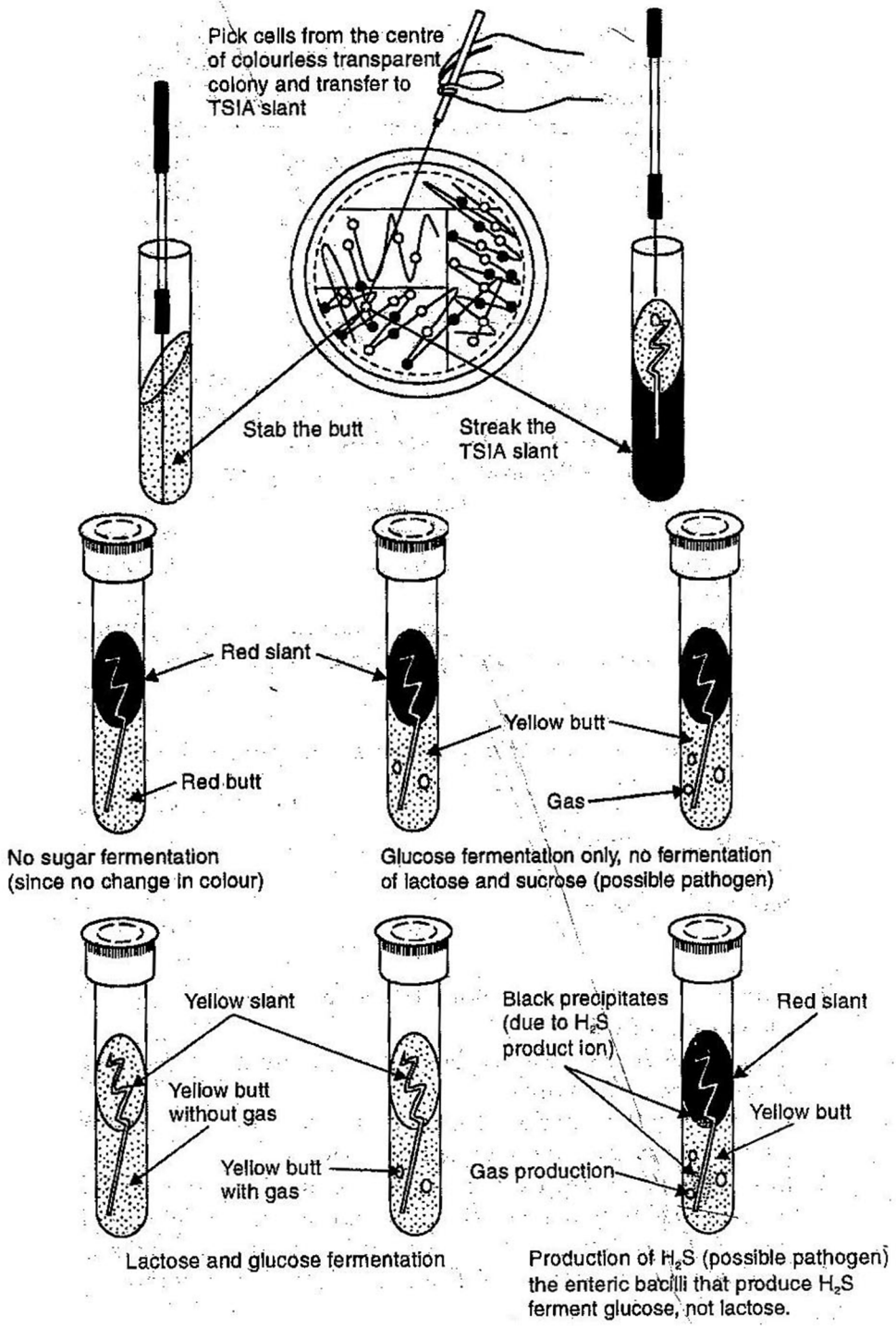


Fig. A.1 Summary of procedure and biochemical reactions in TSIA slants

(Source: Aneja, 2003)

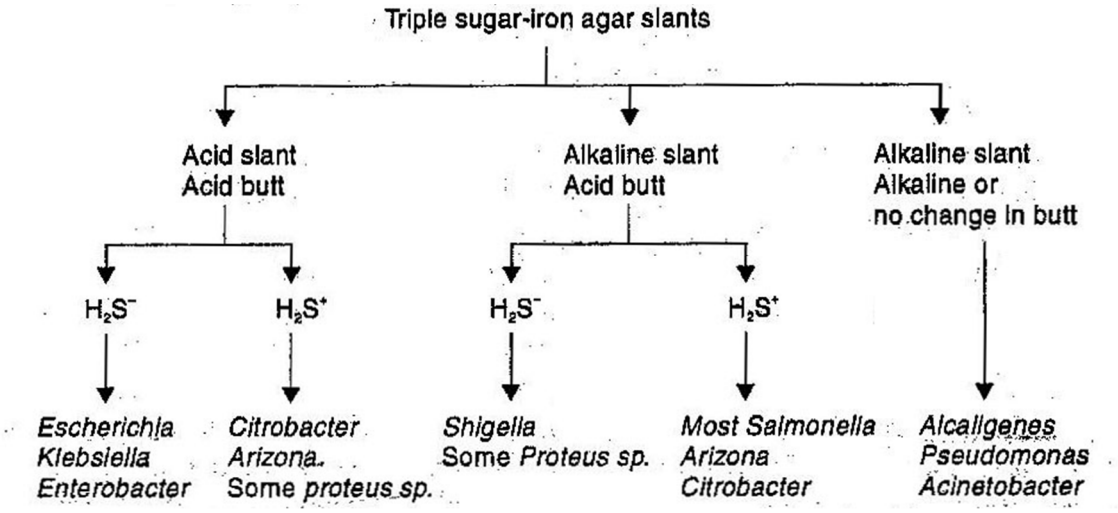


Fig. A.2 Scheme for the differentiation of micro-organisms on the basis of the biochemical reactions on TSIA slants

(Source: Aneja, 2003)

Appendix B

Microbiological criteria for foods

Table B.1 ICMSF guidelines for the assessment of microbiological quality of ready-to-eat foods at point of sale

Test		Microbiological Quality (CFU per gram, unless stated)			
		Satisfactory	**Acceptable**	**Unsatisfactory**	**Unacceptable**
Total Plate Count					
Food Category [a]	1	$<10^3$	10^3 - $<10^4$	$\geq 10^4$	N/A
	2	$<10^4$	10^4 - $<10^5$	$\geq 10^5$	N/A
	3	$<10^5$	10^5 - $<10^6$	$\geq 10^6$	N/A
	4	$<10^6$	10^6 - $<10^7$	$\geq 10^7$	N/A
	5	N/A	N/A	N/A	N/A
Indicators organism (applies to all food categories)					
E. coli (total)		<20	20 - <100	≥100	N/A
Pathogens (applies to all food categories)					
Salmonella spp.		Not detected in 25 gm	N/A	N/A	Present in 25 gm

[a] Category 1 includes beefburgers, meat pies, pork pies, sausage rolls, scotch eggs, raw pickled fish, mousse/desserts. Category 2 includes meat meals, poultry, cakes and pastries (without dairy cream), quiche, mayonnaise, dressings, pasta, *samosa*, vegetables and vegetable meals (cooked), pizza, ice cream, ready-to-eat meals. Category 3 includes sliced beef, sliced pork, sliced poultry, crustaceans, fish, cakes and pastries (with dairy cream), dried fruits and vegetables, rice. Category 4 includes sliced ham, mollusks and other shellfish, prepared mixed salads. Category 5 includes raw ham, smoked sausages, cheesecake, fermented foods, fresh fruits and vegetables, cheese, yoghurt.

N/A denotes “Not applicable”.

(Source: CFS, 2007)

Table B.2 Guidelines for assessing microbiological quality of ready-to-eat foods

Test	Microbiological Quality (CFU per gram)			
	Satisfactory	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Total Plate Count				
Level 1	$<10^4$	$<10^5$	$\geq 10^5$	N/A
Level 2	$<10^6$	$<10^7$	$\geq 10^7$	N/A
Level 3	N/A	N/A	N/A	N/A
Indicators				
*Enterobacteriaceae**	$<10^2$	$10^2 – 10^4$	$\geq 10^4$	
Escherichia coli	<3	3-100	≥100	**
Pathogens				
Coagulase +ve staphylococci	$<10^2$	10^2 - 10^3	$10^3 – 10^4$	$\geq 10^4$ (set +ve)
Clostridium perfringens	$<10^2$	10^2 - 10^3	$10^3 – 10^4$	$\geq 10^4$
Bacillus cereus	$<10^2$	10^2 - 10^3	$10^3 – 10^4$	$\geq 10^4$
Salmonella spp.	Not detected in 25 gm			Detected

Three levels of TPC are listed in based on food type and the processing/handling of food.

Level 1 - applies to ready-to-eat foods in which all components of the food have been cooked in the manufacturing process/preparation of the final food product and, as such, microbial counts should be low.

Level 2 - applies to ready-to-eat foods which contain some components that have been cooked and then further handled (stored, sliced or mixed) prior to preparation of the final food or where no cooking process has been used.

Level 3 - TPC not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods and foods incorporating these.

N/A – Not applicable.

**Enterobacteriaceae* testing is not applicable to fresh fruits and vegetables or foods containing these.

**Pathogenic strains of *E. coli* should be absent.

(Source: Anon, 2001)

Appendix C

Microbiological Criteria for water

Table C.1 Bacterial parameter of water for human consumption

Parameter	Volume of sample (ml)	Guide level	Max permitted no of bacteria
Total coliform	100	-	0
Fecal coliform	100	-	0
Colony count: at 22^{o}C	1	100	No significant increase over that normally observed
at 37^{o}C	1	10	

(Source: K.C. and Rai, 2000)

Table C.2 WHO Guideline values for verification of microbial quality

Parameter	Volume of sample (ml)	Guideline values
Total plate count	1	<10
Total coliform	100	0

(Source: WHO, 1996)

Appendix D

Statistical analysis (ANOVA Tables)

Table D.1 Two way ANOVA for TPC of street food samples

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	3.889×10^{12}	7.779×10^{11}	34.93	<0.001
Place	7	4.757×10^{11}	6.796×10^{10}	3.05	0.013
Residual	35	7.794×10^{11}	2.227×10^{10}		
Total	47	5.145×10^{12}			

Since the value of F pr. is less than 0.05, there is significant difference between the sample types and place of collection of samples for the variate TPC and LSD testing is necessary.

Table D.2 LSD testing for TPC of street food samples (LSD = 151475.8)

Sample	Average	Difference of average	Comments
Dahibada (A)	6.51×10^5	A – B = 29000	<LSD
Chatpate (B)	6.22×10^5	A – C = 478000	>LSD
Panipuri (C)	1.73×10^5	A – D = 643560	>LSD
Pyaji (D)	7.44×10^3	A – E = 646380	>LSD
Aluchop (E)	4.62×10^3	A – F = 650700	>LSD
Momo (F)	3.00×10^2	B – C = 449000	>LSD
		B – D = 614560	>LSD
		B – E = 617380	>LSD
		B – F = 621700	>LSD
		C – D = 165560	>LSD
		C – E = 168380	>LSD
		C – F = 172700	>LSD
		D – E = 2820	<LSD
		D – F = 7140	<LSD
		E – F = 4320	<LSD

Table D.3 LSD testing for TPC of street food collection places (LSD = 174909.2)

Place	Average	Difference of average	Comments
Hatiya (A)	416913	A – B = 79615	<LSD
Tyangra pool (B)	337298	A – C = 137258	<LSD
Paschim line (C)	279655	A – D = 176093	>LSD
Itahari chowk (D)	240820	A – E = 187086	>LSD
Bus park (E)	229827	A – F = 190783	>LSD
Biratnagar line (F)	226130	A – G = 282938	>LSD
Dharan road (G)	133975	A – H = 336560	>LSD
Bhetghat chowk (H)	80353	B – C = 57643	<LSD
		B – D = 96478	<LSD
		B – E = 107471	<LSD
		B – F = 111168	<LSD
		B – G = 203323	>LSD
		B – H = 256945	>LSD
		C – D = 38835	<LSD
		C – E = 49828	<LSD
		C – F = 53525	<LSD
		C – G = 145680	<LSD
		C – H = 199302	>LSD
		D – E = 10993	<LSD
		D – F = 14690	<LSD
		D – G = 106845	<LSD
		D – H = 160467	<LSD
		E – F = 3697	<LSD
		E – G = 95852	<LSD
		E – H = 149474	<LSD
		F – G = 92155	<LSD
		F – H = 145777	<LSD
		G – H = 53622	<LSD

Table D.4 Two way ANOVA for coliforms of street food samples

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	1.041×10^9	2.083×10^8	10.58	<0.001
Place	7	4.086×10^8	5.837×10^7	2.97	0.015
Residual	35	6.889×10^8	1.968×10^7		
Total	47	2.139×10^9			

Since the value of F pr. is less than 0.05, there is significant difference between the sample types and place of collection of samples for variate the coliforms and LSD testing is necessary.

Table D.5 LSD testing for coliforms of street food samples (LSD = 4503.5)

Sample	Average	Difference of average	Comments
Chatpate (A)	1.14×10^4	A – B = 2320	<LSD
Dahibada (B)	9.08×10^3	A – C = 3260	<LSD
Panipuri (C)	8.14×10^3	A – D = 10497	>LSD
Pyaji (D)	9.03×10^2	A – E = 11022	>LSD
Aluchop (E)	3.78×10^2	A – F = 11383.6	>LSD
Momo (F)	1.64×10^1	B – C = 940	<LSD
		B – D = 8177	>LSD
		B – E = 8702	>LSD
		B – F = 9063.6	>LSD
		C – D = 7237	>LSD
		C – E = 7762	>LSD
		C – F = 8123.6	>LSD
		D – E = 525	<LSD
		D – F = 886.6	<LSD
		E – F = 361.6	<LSD

Table D.6 LSD testing for coliforms of street food collection places (LSD = 1811.3)

Place	Average	Difference of average	Comments
Hatiya (A)	11449	A – B = 3681	>LSD
Tyangra pool (B)	7768	A – C = 7054	>LSD
Paschim line (C)	4395	A – D = 7354	>LSD
Bus park (D)	4095	A – E = 7657	>LSD
Itahari chowk (E)	3792	A – F = 7793	>LSD
Biratnagar line (F)	3656	A – G = 8656	>LSD
Dharan road (G)	2793	A – H = 9535	>LSD
Bhetghat chowk (H)	1914	B – C = 3373	>LSD
		B – D = 3673	>LSD
		B – E = 3976	>LSD
		B – F = 4112	>LSD
		B – G = 4975	>LSD
		B – H = 5854	>LSD
		C – D = 300	<LSD
		C – E = 603	<LSD
		C – F = 739	<LSD
		C – G = 1602	<LSD
		C – H = 2481	>LSD
		D – E = 303	<LSD
		D – F = 439	<LSD
		D – G = 1302	<LSD
		D – H = 2181	>LSD
		E – F = 136	<LSD
		E – G = 999	<LSD
		E – H = 1878	>LSD
		F – G = 863	<LSD
		F – H = 1742	<LSD
		G – H = 879	<LSD

Appendix E

Statistical analysis (ANOVA Tables)

Table E.1 One way ANOVA for TPC of water samples

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Place	7	11936.94	1705.28	66.71	<0.001
Residual	8	204.50	25.56		
Total	15	12141.44			

Since the value of F pr. is less than 0.001, there is significant difference between the samples collected from different places for the variate TPC and LSD testing is necessary.

Table E.2 One way ANOVA for coliforms of water samples

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Place	7	947.75	135.39	20.83	<0.001
Residual	8	52.00	6.50		
Total	15	999.75			

Since the value of F pr. is less than 0.001, there is significant difference between the samples collected from different places for the variate coliforms and LSD testing is necessary.

Table E.3 LSD testing for TPC of water samples (LSD =11.66)

Place	Average	Difference of average	Comments
Hatiya (A)	173	A – B = 21.5	>LSD
Paschim line (B)	151.5	A – C = 31	>LSD
Tyangra pool (C)	142	A – D = 42	>LSD
Bus park (D)	131	A – E = 44	>LSD
Biratnagar line (E)	129	A – F = 50	>LSD
Itahari chowk (F)	123	A – G = 80	>LSD
Dharan road (G)	93	A – H = 89	>LSD
Bhetghat chowk (H)	84	B – C = 9.5	<LSD
		B – D = 20.5	>LSD
		B – E = 22.5	>LSD
		B – F = 28.5	>LSD
		B – G = 58.5	>LSD
		B – H = 67.5	>LSD
		C – D = 11	<LSD
		C – E = 13	>LSD
		C – F = 19	>LSD
		C – G = 49	>LSD
		C – H = 58	>LSD
		D – E = 2	<LSD
		D – F = 8	<LSD
		D – G = 38	>LSD
		D – H = 47	>LSD
		E – F = 6	<LSD
		E – G = 36	>LSD
		E – H = 45	>LSD
		F – G = 30	>LSD
		F – H = 39	>LSD
		G – H = 9	<LSD

Table E.4 LSD testing for coliforms of water samples (LSD = 5.879)

Place	Average	Difference of average	Comments
Tyangra pool (A)	48	A – B = 4	<LSD
Hatiya (B)	44	A – C = 5	<LSD
Paschim line (C)	43	A – D = 9	>LSD
Bus park (D)	39	A – E = 11	>LSD
Itahari chowk (E)	37	A – F = 13	>LSD
Biratnagar line (F)	35	A – G = 17	>LSD
Dharan road (G)	31	A – H = 26	>LSD
Bhetghat chowk (H)	22	B – C = 1	<LSD
		B – D = 5	<LSD
		B – E = 7	>LSD
		B – F = 9	>LSD
		B – G = 13	>LSD
		B – H = 22	>LSD
		C – D = 4	<LSD
		C – E = 6	>LSD
		C – F = 8	>LSD
		C – G = 12	>LSD
		C – H = 21	>LSD
		D – E = 2	<LSD
		D – F = 4	<LSD
		D – G = 8	>LSD
		D – H = 17	>LSD
		E – F = 2	<LSD
		E – G = 6	>LSD
		E – H = 15	>LSD
		F – G = 4	<LSD
		F – H = 13	>LSD
		G – H = 9	>LSD

Appendix F

Table F.1 Microbiological quality of some street foods found at Biratnagar market

Sample	No of obs.	TPC (cfu/g)	Coliforms (cfu/g)
Chatpate	1	3.43×10^{3}	5.07×10^{3}
	2	1.16×10^{5}	3.77×10^{4}
	3	9.87×10^{3}	8.27×10^{3}
Dahibada	1	6.85×10^{3}	1.31×10^{3}
	2	1.85×10^{5}	1.20×10^{4}
	3	5.96×10^{4}	7.01×10^{3}
Alubhujiya	1	9.06×10^{3}	2.27×10^{2}
	2	2.67×10^{4}	4.59×10^{3}
	3	7.63×10^{3}	6.58×10^{3}
Momo	1	4.3×10^{1}	Nil
	2	5.73×10^{2}	3.4×10^{1}
	3	1.71×10^{2}	Nil
Chaumin	1	4.64×10^{3}	5.0×10^{1}
	2	1.01×10^{2}	Nil
	3	3.17×10^{2}	7.9×10^{1}
Panipuri	1	4.74×10^{3}	6.56×10^{3}
	2	3.46×10^{4}	2.54×10^{4}
	3	1.23×10^{3}	8.41×10^{3}

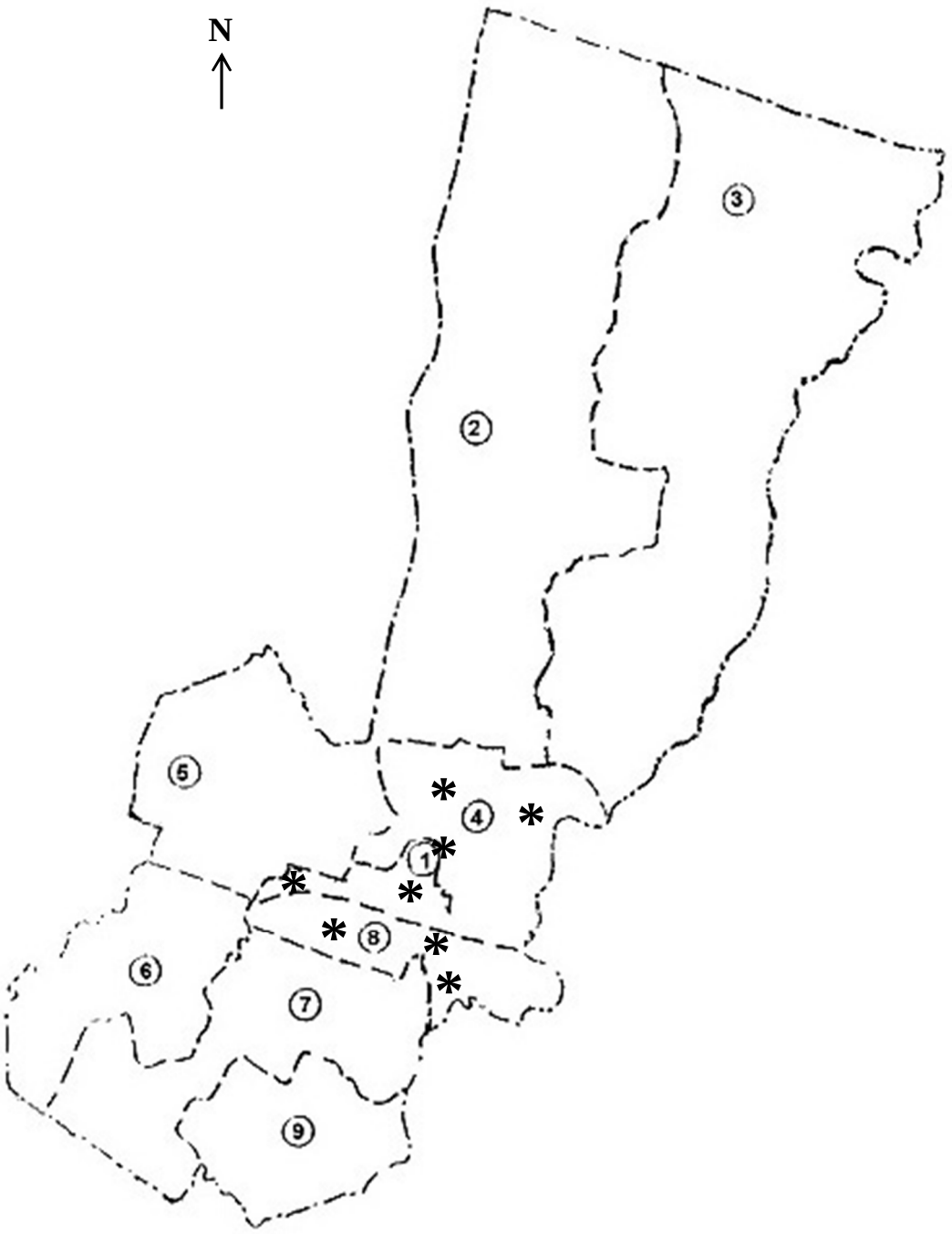
(Source: Karki, 2005)

Table F.2 Microbiological quality of some street foods found at Dharan market (n = 12).

Food Items	TPC (cfu/g)	Coliforms (cfu/g)
Chatpate	$5.7\text{x}10^{6}$ - $1.1\text{x}10^{8}$	$1.0\text{x}10^{4}$ - $9.3\text{x}10^{4}$
Dahibada	$1.8\text{x}10^{7}$ - $1.2\text{x}10^{8}$	$1.0\text{x}10^{4}$ - $9.0\text{x}10^{4}$

(Source: Dahal, 1993)

Appendix G



Legends:

— — — Ward border

—·—·— Municipality border

* Sampling site

Fig. G.1 Map of sampling site