CHAPTER – I
INTRODUCTION

1.1 Background

Nowadays, the consumption of meat and meat products is increasing with increasing population, economic growth, urbanization, modernization and industrialization. Meat and meat products are regarded as high nutritive value food. A typical meat contains about 20% protein, 70% water, 5% lipid, and 5% other substances example carbohydrate, salts, vitamin, etc (Nakai and Modler, 2000). Meat is an excellent source of long chain omega 3 polyunsaturated fats, riboflavin, pantothenic acid, selenium and vit D. It is also low in fat and sodium. Meat and meat products are also an excellent source of high quality animal protein, vitamins especially B complex, and certain minerals, especially iron (Gracey *et al*., 1986).

The bacterial flora on fresh meat contains around 30 different genera and the most common microflora of fresh meat is composed primarily of:

1. Gram negative aerobic cocci and rods:- *Aeromonas* spp, *Enterobacter* spp, *Citrobacter, Escherichia, Proteus, Salmonella, and Campylobacter Pseudomonas, and Acinetobacter*
2. Gram positive rod:- *Clostridium* spp, *Bacillus*, *Brochothrix* spp, *Lactobacillus and Listera* spp
3. Gram positive cocci:- *Pedicococcus, Enterococcus* spp, *Lactococus* spp, *Micrococcus and Staphylococcus.*

Meat and meat products are considered as an ideal culture medium for growth of many organisms because of the high moisture, high percentage of nitrogenous compounds of various degree of complexity, plentiful supply of minerals, accessory growth factors and some fermentable carbohydrates (glycogen) of a favorable pH for most of the enteric microorganisms (Frazier and Westhoff,

1978). Food products (including meat) are contaminated with soil, air and waterborne micro-organisms during harvesting, processing, distribution and preparation. Extremely high numbers of micro-organisms are found in meat animals intestinal tracts, and some of these find their way to the carcass surfaces during slaughter. Some apparently healthy animals may harbour various micro-organisms in the liver, kidneys, lymph nodes and spleen. These micro-organisms and those from contamination through slaughtering can migrate to the skeletal muscles via the circulatory system (Marriot, 1994).

Meat is the indispensable food in our daily life but it's contaminated easily by the microorganism because of its rich nutrient content. Microbial ecology of meat product will mainly depend on the environment, kind of meat, and raw material, equipment, handling practices, processing, packing, and storage temperature (Sachindra *et al.,* 2003). Food spoilages microorganism are responsible for detrimental quality changes in meat. The changes include discoloration, unpleasant odors and physical alteration. The principal spoilage organisms are molds and bacteria. Mold can impart a musty flavor to meat. Common mold meat includes the genera *Cladasporium*, *Mucor* and *Alternaria.* Slime molds produce a soft, creamy material on the meat. Common spoilages bacteria include *Pseudomonas*, *Acinetobacter* and *Morexella*. Under anaerobic condition, such as in canned meat spoilage include souring, putrefaction and gas production.

Microbial contaminant rather common than any other form of contaminant as food animal itself harbor them. Microbial status of fresh meat depend on animal rearing, transportation, slaughtering and cutting and packing, besides hygiene and processing conditions of the slaughter plant. The natural surfaces flora of meat animals usually is not important as the contaminating microorganism from their intestinal or respiratory tracts. However hides, hooves, and hair contain not only larges number of microorganism from the soil, manure, feeds, and water but also important kind of spoilages organisms. Bacteria present in the muscle fibers and other parts of the care may be due to slaughtering practices or infection of the

animal prior to slaughter, such as *Brucella*, *Salmonella*, *Streptococcus*, and *Mycobacterium tuberculosis*, also certain anaerobic bacteria may be present. *Achromobacter* and *Pseudomonas* are predominant in meat held at temperatures. Also the presence of Bacilli, Staphylococci, and lactobacilli may contribute to surface slime (Biswas, 2011).

Sausages are defined as a diverse group of foods made from ground or comminuted meats, salts, and spices and packed into natural casing consisting of the connective tissue and muscles tissue of animal intestines and artificial casing (made up of cellulose collagen of synthetic material. They are originated during pre historic time when our ancestors discovered that the addition of salt and drying would delay spoilage. Sausages continued to have great popularity because of their appealing flavor and convenience (Ockermar, 1989).

Sausages are usually meat based. Sausages are excellent sources of high quality proteins. In addition, they are also a good source of iron, Zn and B vit particularly folic acid, B6 and B12 because of essential nutrients they contain, sausages can be regarded as a valuable component of balanced diet and can alleviate common nutritional deficiencies (Rust, 1987).

Meat products due to its nutritive value are favored by the microorganism including many pathogens. Microorganism set into the food by raw materials, water, unclear cooking utensils, and environmental contamination and by the people handling the food in its preparation and sale. Sausages may get contaminated due to inferior quality of raw meat, lack of adequate potable water for the preparation and cleaning of equipments and utensils and may be due to poor personal hygiene and environmental condition of processing room.

In Kathmandu Valley, the population is increasing rapidly resulting in an increasing problem with regard to sanitation, hygiene, availability of clean drinking water and food hygiene. Consumption of milk, meat and eggs is also

increasing rapidly but has not been accompanied by proper marketing practices (Joshi *et al.*, 2003).

In Nepal road, bank of the river, ground floor aside the dusty road are used for the slaughtering and selling the meat rather than the scientifically designed slaughter houses. One of the main sources of the contamination is the dirty water used for spraying the animal before slaughtering and cleaning after slaughtering. Due to the scarcity of clean water in the Kathmandu, butcher use ground water for various meat processing processes. There could be possible cross contamination between adjacent raw meat through unclean hands of the handlers and/ or flies. Careless sneezing and coughing with butchers, handling the carcasses and the money with the same unwashed hands could be good sources of contamination of the product (Joshi *et al*., 2003).

Meat is an important source of protein and a valuable commodity in resource poor communities. In many developing countries like Nepal, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses of meat as well as invaluable by-products from animal carcasses. Slaughtering places are frequently contaminated and may not be protected against dogs, rodents and insects. Meat products coming from such conditions are often deteriorated due to bacterial infection or contaminated, which may cause food poisoning or diseases in consumers. In many developing countries, regulations concerning meat inspection and/ or control are inadequate or non-existent allowing consumers to be exposed to pathogens including zoonotic parasites.

In Nepal, about 64% of the meat consumed is of buffaloes, followed by goat meat (20%), pork (7%), poultry (6%) and chevon (2%). Due to the lack of implementation of the Meat Inspection Act and resultant absence of meat inspection, meat from sick or parasite ingested and infected animals is serving as a source of infection to humans as well as other animals. Beside this, meat quality

is adversely affected by careless handling conditions in the slaughtering places as well as in the meat markets or shops (Joshi, 2003).

In addition to that fresh meats are sold along with the animals gut everyday at a retail level to the public on the shop. Fresh meat is always kept openly while awaiting the buyers, making it naturally vulnerable to infection with different types of microorganism. Therefore, improper handling and improper hygiene may lead to the contamination of fresh meats and eventually affects the health of the consumers (Kousseman *et al*., 2008).

1.2 Objectives

General objective

The general objective of the study is to assess the microbiological quality of the raw buff meat and raw buff sausages sold in Bhaktapur.

Specific objectives:

a. To enumerate the total bacterial load of buff meat and buff sausages

b. To determine the total coliform count of buff meat and buff sausages

c. To identify the organisms from the buff meat and buff sausages

d. To assess the antibiotic susceptibility test of isolated bacteria from the raw buff meat and raw buff sausages.

CHAPTER – II
LITERATURE REVIEW

Our basic needs include air that contain adequate oxygen, water that is potable, edible food and shelter. Food provides us energy needed for work and for various chemical reactions. Food is also necessary for our existence that the search for the food has been the main occupation of human beings throughout history (Banwart, 1987)

Meat and meats products are indispensable food consumed by most of the people of this universe. The consumption of meat and meat products is increasing with the increasing population. The food from the plant origin provides moderate level of vitamin, protein and minerals. On the contrary food of animal origin or meat is a good source of protein, vitamin, vit B6, vit B12, folic acid, niacin, essential fatty acids and mineral likes zinc, iron etc Most meat for human consumption comes from domestic animals, including cattle, pigs, sheep, chickens, turkeys, ducks and rabbits. Meat is a nutritious food as the protein provides all essential amino acids in the proportionate amounts required by man and is also an excellent source of iron, thiamine and niacin, phosphorus, potassium and sodium (Schonfeldt & Welgemoed, 1996). Meat is an important food in the mountain region; where it is scarcely possible to grow field crops; ruminants and other herbivores can be used to convert vegetation into high quality food stuff as meat.

2.1 Microorganisms found in meat

The microbiological condition of carcass meat is highly dependant on the manner, in which meat animals are reared, slaughtered and processed. It is important that only relatively clean animals are presented for slaughtering, since it is extremely difficult to obtain clean meat from dirty animals. Therefore, the cleanliness of livestock depends on husbandry, weather and climate, methods of transport and holding conditions at the abattoir.

Jay (1992) explains that comminuted meats such as ground beef invariably have higher numbers of micro-organisms than non-comminuted meats such as steaks. Commercial ground meats generally consist of trimmings from various cuts. These pieces have been handled excessively and consequently normally contain more micro-organisms than meat cuts such as steaks. Ground meat also provides a greater surface area, which itself accounts in part for the increased flora. This greater surface area of ground meat favors the growth of aerobic bacteria, the usual low-temperature spoilage flora. One heavily contaminated piece of meat is sufficient to contaminate others, as well as the entire lot, as they pass through the grinder. This heavily contaminated portion is often in the form of lymph nodes, which are generally embedded in fat. Psychrotrophic strains of *Achromobacter, Micrococcus, Flavobacterium* and *Pseudomonas,* were recovered from the carcasses after dressing. Psychrotrophic bacteria, the group that includes potential spoilage bacteria for chilled meat, are common in soil, water and vegetation (Newton, Harrison and Wauters, 1978).

Nortje and Naude (1981) point out that the most commonly encountered bacteria on fresh meat are *Pseudomonas* spp, *Moraxella* spp, *Acinetobacter* spp, *Microbacterium thermosphactum* and members of the Enterobacteriaceae. However, members of the bacterial genera *Aeromonas, Alcaligenes, Arthrobacter, Brochothrix, Brucella, Campylobacter, Corynebacterium, Enterobacter, Escherichia, Flavobacterium, Hafnia, Listeria, Microbacterium, Pediococcus, Salmonella, Staphylococcus* and *Yersinia* can be recovered from red meat and their products to a lesser extent (Weizer, Mounty and Gould, 1978). Of particular concern are the pathogenic mesophiles such as Salmonella, which have been observed to grow on meat at 25 °C (Gill and Newton, 1980).

The healthy inner flesh of meats has been reported to contain few or no micro-organisms, although they have been found in lymph nodes, bone marrow, and even flesh. Staphylococci, Streptococci, Clostridia and *Salmonella*, have been isolated from the lymph nodes of red-meat animals. The important contamination, however, comes from external sources during bleeding, handling and processing.

During bleeding, skinning and cutting, the main sources of micro-organisms are the exterior of the animal (hide, hooves and hair) and the intestinal tract. Approved “humane” methods of slaughter mechanical, chemical and electrical have little effect on contamination, but each method is followed by sticking and bleeding, which can introduce contamination (Bekker, 1998; Frazier and Westhoff, 1988).

2.2 Sources of Micro-organisms in Meat

Since the animal’s body is not free of bacteria and since, after death bacteria can enter the tissues through the intestinal and other mucosa, the carcass may have an inherent microflora.

The surface of meat may contain a variety of micro-organisms primarily to air contamination. No particular kinds of organisms are inherent on the surface of the meat.

Bacteria present in the muscle fibers and other parts of the care may be due to slaughtering practices or infection of the animal prior to slaughter, such as *Brucella, Salmonella, Streptococcus,* and *Mycobacterium tuberculosis,* also certain anaerobic bacteria may be present. *Achromobacter* and *Pseudomonas* are predominant in meat held at temperatures. Also the presence of *Bacilli, Staphylococci,* and *lactobacilli* may contribute to surface slime.

2.3 Factors that influence microbial contamination of meat

1. Bacterial load in gut. Starvation for 24 hours recommended.
2. Physiological changes:
    Animal excited or fatigued, bacteria enter tissue more readily.
    Incomplete bleeding.
    Glycogen used up in fatigue, pH will not drop to 5.5 from 7.2.
3. Method of killing and bleeding.
4. Rate of cooling.
5. Grinding meat influences bacterial count.

2.4 Sources of contamination of meat

The exterior surfaces (hide, hair, skin) of healthy live animals are naturally contaminated with large numbers (10^7 organisms per cm^2 of hide) of a variety of organisms (Featherstone, 2003; Greer and Jeremiah, 1980). Slaughter stock themselves are therefore a major source of carcass contamination. The hide or intestinal tracts of slaughtered animals are the main areas where potentially pathogenic and spoilage bacteria reside. The soil is also a major source of micro-organisms and has comparable numbers (10^7) of bacteria per gram of soil. Faeces are about 100 times more contaminated and have an aerobic plate count and coliforms of about 10^9 and 10^8 per gram of faeces, respectively. It can therefore be said that all of these can serve as sources of microbial contaminants of the meat.

The instruments used in dressing and killing (knives, saws, cleavers and direct contact with hides and hair as well as by contact with steels, knife scabbards and the clothing of operatives and hooks), various vessels, receptacles and the personnel may all act as sources of contamination during slaughter (Lawrie, 1985; Thornton and Gracey, 1974; Kirkpatrick, 2002).

The following are the primary sources and route of microorganism to fresh meats.

The stick knife:- After being stunned and hoisted by the hind legs, animal such as steers are exsanguinated by slitting the jugular vein with what is referred to as a "stick knife". If the knife is not sterile, organisms are swept into the blood stream, where they may be deposited throughout the carcass (Jay, 1987).

Animal hides:- Organism from the hides is among those that enter the carcasses via the stick knife. Other from the hide biota becomes airborne and can contaminate dressed out carcasses. The hide remains an important source of micro-organisms for contamination of the carcass (Jay, 1992).

Gastrointestinal tract:- The intestinal contents contain intestinal organism or intestinal flora which may gain accesses into the freshly dressed carcass through

the punctures. Especially important in this regard is the rumen of the ruminant animal which typically contains nearly 10^{10} bacteria per gram.

Hands of handlers:- Hands of handlers is a sources of human pathogens to the freshly slaughter meat. People working in meat processing plant also can act as vector of many food borne pathogenic bacteria (Frazier and Westhoff, 1999). According to Marriot (1994), employees are the largest contamination source and employees who do not follow sanitary practices, contaminate food that they touch with spoilage and pathogenic micro-organisms. Employees come in contact with these micro-organisms through work and other parts of the environment while their hands, hair, nose and mouth, harbour micro-organisms that can be transferred to food during processing, packaging, preparation and service by touching, breathing, coughing or sneezing. Therefore, in the prevention of meat contamination, personal hygiene plays an important role as there are as many as 200 different species of micro-organisms on a healthy human body (Hobbs and Roberts, 1993; Featherstone, 2003).

Containers:- Meats cut that are placed in the nonsterile containers may be expected to become contaminated with the organism in the container. Further, the equipment used in the slaughtering and dressing operations (knives, saws, cleavers and hooks) make significant contributions to the overall contamination through direct contact with hides and hair as well as by contact with steels, knife scabbards and the clothing of operatives (Thornton and Gracey, 1974).

Handling and storages environments:- Pelczar, Chan and Krieg (1986) are of opinion that: “The carcass of a healthy animal slaughtered for meat and held in a refrigerated room is likely to have only nominal surface microbiological contamination while the inner tissues are sterile”. Contamination occurs by the introduction of micro-organisms on the meat surfaces in operations performed during cutting, processing, storage, and distribution of meat. Generally, contamination occurs when the meat comes into contact with dirty hands,

clothing, equipment and facilities (Lawrie, 1985; Frazier and Westhoff, 1988; Trickett, 1997).

Lymph node:- Lymph node contains high numbers of micro-organisms and is embedded in fat. If they are cut through or added to the portions that are ground, one may expect this biota to become prominent.

2.5 Spoilage of the Buff Meat

Usually, fresh cut meats in the refrigerator at high humidity undergo bacterial spoilage by: Gram negative aerobes like *Pseudomonas, Acinetobacter* and *Moraxella* spp. The intrinsic and extrinsic parameters of ground beef favor these bacteria so strongly that they are almost exclusive spoilage agents. Meat spoilage is characterized by the appearance of off odors and slime, which is manifest when surface loads exceed 10^7 CFU/cm^2.The slime is due to the accumulation of bacterial cells. Meat spoilage (including poultry and fish) occurs without any significant breakdown of the primary protein structure. Instead, spoilage bacteria utilize glucose, free amino acids or other simple nitrogenous compounds to attain population of about 10^8 CFU/cm^2, at which point the organoleptic quality of the meat will clearly reveal it is spoiled.

Meats may undergo spoilage as a result of microbial action on the fats and proteins. Many organisms produce the enzyme lipase which attacks the fat. Thus the presence of these micro-organisms in meat is significant in that they may bring about more or less rancidity. Also, other microorganisms may form enzymes capable of producing significant changes in the protein. Oxidizing organisms sometimes impart tallowy flavors to meats containing fat. Many kinds of organisms are able to initiate hydrolysis of fats, but only a few varieties can oxidize fats directly.

The main forms of sausage spoilage and deterioration are the excessive proliferation of bacteria in the sausage content or on the surface, the excessive growth of moulds on the sausage surface, the oxidative deterioration of sausage

fat causing product rancidity, and the excessive dehydration of sausage superficial layers including casings.

The rates at which these four forms of spoilage and deterioration can occur vary widely. For example, processing under unhygienic conditions may cause souring, gas formation, off-odours etc. within a few hours after production or the spoilage process can be somewhat delayed and will develop during a longer time period, perhaps in the consumer's home. High storage temperatures and high humidity, poor handling and other adverse conditions may similarly accelerate bacterial and fungal development, especially on the surface of the products. On the contrary, dry air atmosphere, high temperatures and particularly high air circulation rates contribute essentially to development of rancidity and surface dehydration, often accompanied by discoloration and other organoleptic changes.

2.6 Preservation

The storage life of ground beef that contains 1 million bacteria per gram is approximately 28 hours at 15.5 °C. At a normal refrigerated storage temperature of approximately -1 to 3 °C, the storage life exceeds 96 hours (Marriot, 1994). Shelf life is therefore obviously influenced by the initial load of contaminating microorganisms.

Meat is the most perishable food of all major food. Most meat are very good culture medium-high in moisture, neutral in pH and high in nutrient-coupled with the fact that some organism may be found in lymph nodes, bones, and muscles and contaminate with spoilage organism is almost unavoidable makes the preservation of meat more difficult than that of the most kinds of food (James, 1996).

2.6.1 Asepsis

Asepsis meaning keeping away from the microorganism is one of the easier mean of preservation of meat (Frazier and Westhoff, 1988).

2.6.2 Uses of low temperature

Two method of preserving meat through low temperature namely chilling and freezing can be applied. For chilling meat is stored at the temperature of 0^0C to 4^0C and for freezing at -18^0C. The carcass of healthy animal slaughter for meat and held in refrigerated room is likely to have nominal surface microbial contamination (Pleczar Chan and Krieg, 1986). The main reason for chilling meat is to control the proliferation of bacteria and certain other microbes such as yeast and moulds on meat and to reduce the rate of deteriorative chemical changes e.g. oxidation of fats causing rancidity (Frazier and Westhoff, 1988). Further, by means of chilling the shelf life of meat is lengthened by slowing down the multiplication of organisms, which cause meat to spoil, and which cause food poisoning.

2.6.3 Use of irradiation

Irradiation with UV rays in conjunction with chilling storage can increase the shelf life of the meat by reducing the number of microorganism in the air and inhibiting or killing the microorganism on the surface of the meat reached directly by the rays.

2.6.4 Preservation by drying

Meat and meat products such as dry sausages, dry salamis and dry cervelals are preserved by drying. Drying lower the available water content of the meat hence control the growth of microorganism. Freezing, drying where foods are frozen and water is removed under vacuum of the meat is on increase.

2.6.5 Curing

Various types of curing agents like sodium chloride, sugar, sodium nitrate and vinegar commonly used for the preservation of meat.

2.6.6 Smoking

Preservative substances added to the meat, together with the action of heat during smoking, have a germicidal effect and that drying of the meat together with chemical from the smoke, inhibit the microbial growth during storage.

2.7 Food borne pathogen associated with buff meat

Foods of animal origin are the primary source of many bacteria responsible for food borne infection and intoxication. Raw meat is important sources of *Salmonella* and *Cl. perfringens* which are often incriminated in the outbreaks of foodborne diseases (ICMSF, 1980**)**. Bacterial food poisoning is widely spread and occurs when our environments are untidy and the foods are hygienically maintained. Fresh meats are sometimes contaminated with bacteria which are harmful to the human body. The major bacterial pathogens include: *Salmonella, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Bacillus cereus* and *Escherichia coli*. The sources of these microbes in meat could be inherent microflora in normal tissues of animals, air, environment or contamination due to unhygienic slaughtering, handling and processing conditions.

2.7.1 *Clostridium perfringens*

It is ubiquitous and although it occurs on carcass meat, usually in low numbers, it can't be controlled by any known means. The majority of outbreak of *Cl. perfringens* gastroenteritis attributable to the meat result from inadequate storage cook product. Prevention involves attention to the time and temperature condition of cooking and more important to hot holding, cooling and reheating before

consumption.The enterotoxin produced by *Cl. perfringens* during sporulation of vegetative cells in the host intestine results in debilitating acute diarrhea and abdominal pain.

2.7.2 *Staphylococcus aureus*

S. aureus is a gram positive coccus, resistant to heat, drying, and radiation. Its strains can be pathogenic and relatively non pathogenic. They produce disease when the bacteria contaminate food. They produce some enzymes which are implicated with staphylococcal invasiveness and many extracellular substances some of which are heat stable enterotoxins that render the foods dangerous even though it appears normal (Prescott *et al*., 2005). *S. aureus* may occur in raw meat although usually in low number. The most common symptoms of Staphylococcal food poisoing are nausea, vomiting, retching, abdominal cramping, and prostration. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur.

2.7.3 *Camplylobacter jejuni*

Campylobacter is often present in the intestinal flora of the healthy animals used for food production. However the number presents in red meat are generally low and the organism has only a limited potential for growth or survival on refrigerated or cooked meat. Symptoms of food poisoning from *Campylobacter* usually occur 2 to 5 days after a person eats contaminated meat, but may take up to 10 days to appear. The most common symptom of a *Campylobacter* infection is diarrhea, which is often bloody. Typical symptoms include: diarrhea: diarrhea ranges from mild to severe and is often bloody, fever, nausea, vomiting, abdominal pain, headache, muscle pain.

2.7.4 *Bacillus cereus*

Although most *Bacillus* spp is harmless, a few are pathogenic to humans and animals. B. *cereus* is a normal soil inhabitant, and is frequently isolated from a variety of foods, including vegetables, dairy products and meat. *B. cereus* causes food poisoning similar to staphylococcal food poisoning. Two types of illness have been attributed to the consumption of food contaminated with *B. cereus*. The "diarrhoel syndrome" is characterized by abdominal pain and diarrhea. It has an incubation period of 8 to 16 hours and symptom last 12 to 24 hours. The "emetic syndrome" is characterized by an acute attack of nausea and vomiting, which occurs 1 to 5 hours after a meal (Frazier *et al*., 1997).

2.7.5 *Escherichia coli*

It is gram negative motile bacilli, forms gas from glucose, ferments lactose, produces indole, gives a positive methyl red reaction and a negative Voges Proskauer reaction and does not utilize citrate, grown in KCN, decompose urea or liquefy gelatin (Collee *et al*., 1996).

There are six major categories of *E. coli* strains that cause enteric diseases in humans, including the:

1. enterohemorrhagic *E. coli*, which cause hemorrhagic colitis and hemolytic uremic syndrome,
2. enterotoxigenic *E. coli*, which induce traveler's diarrhea,
3. enteropathogenic *E. coli*, which cause a persistent diarrhea in children living in developing countries,
4. enteroaggregative *E. coli*, which provokes diarrhea in children,
5. enteroinvasive *E. coli* that are biochemically and genetically related to *Shigella* species and can induce diarrhea.

2.7.6 *Listeria monocytogenes*

Listeria monocytogene is Gram-positive foodborne bacterial pathogen and the causative agent of human listeriosis. Listeria infections are acquired primarily through the consumption of contaminated foods, including soft cheese, raw milk, deli salads, and ready-to-eat foods such as luncheon meats and frankfurters. Although *L. monocytogenes* infection is usually limited to individuals that are immunocompromised, the high mortality rate associated with human listeriosis makes it the leading cause of death among food borne bacterial pathogens.

2.7.7 *Salmonella* spp

Salmonella is gram negative, generally motile rod. It is fermentative, facultatively anaerobic, oxidase negative, non lactose fermenting, urease negative, citrate utilizing, acetyl-methyl carbinol negative and KCN negative organism. It is non acid fast, non capsulated and non sporing organism causing serious illness like typhoid fever and salmonellosis (Cheesbrough, 1993).

2.7.8 *Shigella* spp

Shigella spp is Gram-negative, non-spore-forming, non-motile, rod-like members of the family Enterobacteriaceae, which grow in the presence or absence of oxygen. Shigellosis is an acute gastrointestinal disease of humans, caused by four species or serogroups of the genus *Shigella*: *S. dysentariae* (group A), *S. flexneri* (Group B), *S. bodii* (Group C) and *S. sonnei* (Group D). *Shigella* invades the intestinal mucosa producing dysentery characterized by abdominal pain, fever and diarrhea. Symptoms include mild to severe diarrhea with or without blood, fever, tenesmus and abdominal pain.

2.8 Research work carried out

Duitschaever *et al.* (1973) found total aerob mesophiles and psycrotrophic bacteria in 64% of ground meat samples with the counts more than $>10^6$ cfu/g. Staphylococci spp in 98 % samples with numbers more than $>10^3$ cfu/g. Enterococci spp ranged from 1.0×10^1 to 10^4 cfu/g. Coliform was found in 95 % samples at the average numbers of 1.0×10^2 cfu/g. 17% samples contained coagulase (+) Staphylococci spp. No *Salmonella* spp was isolated.

Tekinsen *et al*. (1980) examined 20 samples and found that total aerob mesophiles, psychotrophic bacteria, fecal Streptococci spp, Staphylococci spp, coliform, *E. coli,* bacteria that capable of reducing sulphide and *Cl. perfringens* were counted at the average levels of 8.4×10^7 , 6.2×10^7 , 1.5×10^5 , 9.6×10^5 , 8.5×10^6 ,4.2×10^6 , 6.7×10^3 and 3.9×10^2 cfu/g respectively.

Nortje *et al. (*1989) studied on microbiological quality of retail beef meat. They found that the good hygiene at the retail level was reflected in lower counts for all group of bacteria and greater shelf life. They also found that aerobic count was generally greater for minced meat.

Zhao *et al.* (1998) performed an experiment on cross contamination. They develop a laboratory model to determine occurrence of cross contamination. *Enterobacter aerogens* B 199a, an indicator bacterium with attachment characteristics similar to that of *Salmonella* spp was used. Chicken meat with skin inoculated with 10^6 CFU of *Enterobacter aerogenes* B 199A /gm was cut into small pieces on a sterile cutting board. The extent of cross contamination occurring from meat to the cutting board and from cutting board to vegetable (lettuce and cucumbers) subsequently cut on the board was determined. Swab sample from the cutting board, hand washings, and lettuce and cucumber samples reveled that 10^5 CFU of *E. aerogens*/cm^2 were transferred to the board and hands and approximately 10^3 to 10^4 CFU of *E. aerogenes*/gm to the lettuce and

cucumbers. The result indicate that bacteria with attachment characteristic similar to *Salmonella* spp can be readily transmitted to cutting boards during food preparation and the cross contaminate fresh vegetables if the boards are not cleaned.

Banwart (1987) cross contamination of food is one of the 10 main factors that contribute to food borne illness. People handling both cooked and raw foods can transfer microorganism from the raw to cooked product. With no further treatment, this is a potential health hazard. The housewife who cuts up the raw vegetables for salad may transfer *Salmonella* spp from the raw chicken to the raw vegetables. The meat department in a retail store may use the same knife and block to cut fish, cold meat, chicken, beef and other types of food. It is evident that a potential health hazard can result.

According to James M. Jay (1987) the saw blade may be the major sources of cross contamination as the saw blade had significant counts of total log/in^2 count of 5.28, with 2.3 coliform, 3.64 enterococci, 1.60 staphylococci, and 3.69 micrococci. The cutting block had a mean log/in^2 count of 5.69, with 2.04 coliform, 3.77 enterococci, <1.00 staphylococci and 3.79 micrococci. These are among the sources of high total bacterial count to comminuted meats. Moreover, one heavily contaminated piece of meat is sufficient to contaminate others as well as the entire lots as they pass through the grinder.

Spoilage of meat and meat products due to microorganisms is well known. In some cases the organism are known to have beneficial effect by partially converting the raw material into more palatable substances. But more often they cause heavy loss to meat. Meat and meat products are exposed to various types of microbial contaminants, but only certain types of organisms seem to establish quickly and cause sever damage. Bacteria particularly more dangerous in this respect *Pseudomonas* spp, *Leuconostoc* spp, *Bacillus* spp, *Micrococcus* spp, *Flavobacterium* spp, *Chromobacterium* spp etc are some of the genera associated

with meat spoilage. Some of the psychrophilic and mesophilic organism are known to cause severe damage to meat and meat products stored under low temperature. A kind of the food poisoing is caused by strains of *Cl. perfringens* the food commonly involved is cooked meat left overnight at room temperature and eaten next day (Rangaswami *et al*., 1996)

Khalafalla *et al.* (1993) examined 10 ground beef meat samples and total aerob mesophiles, enterobacteriaceace and staphylococci spp were found at the levels of 10^6, 10^4 and 10^3 CFU/gm respectively.

The effects of freezing thawing and frozen storage on microbial profile of buff meat were studied. It was found that a reduction in microbial count during frozen storages. Coliform were highly sensitive, where as staphylococci and moulds were resistance to frozen storage. It ws also found that micrococci were most predominant, followed by Staphylococci, *Pseudomonas* spp and Bacilli at the end of storage period (Ziauddin *et al.*, 1993)

Davidson *et al.* (2000) reported coliform and *E. coli* at the level of $1.2x10^4$ and $4.8x10^3$ respectively. *Salmonella* spp was isolated from six samples out of 47.

Prasai (2000) examined 14 raw buff meat sample of Kathmandu valley and showed that the total plate counts ranged from $2.2x10^5$ to $2.98x10^7$ cfu/gm and coliform count ranged from $1.3x10^4$ to $1.1x10^6$ cfu/gm and the presence of the coliform with 14 different kinds of enteric bacteria with the highest recovery of *E.coli* (14%), followed by *Bacillus* spp and *S. aureus* (14%), *Pr. vulgaris* (9.9%), *P. aeruginosa, Enterobacter* spp and *Salmonella* spp (7.7%), *K. oxytoca* (6.6%), *Streptococcus faecalis* (5.5%), *C. diversus* (4.4%), *Providencia rettgeri*, *C. freundii*, and *Pr. mirabilis* (3.3%) and *E. cloacae* (2.2%).

Prasai (2000) assayed 15 antibiotics against the total of 281 isolaes isolated from raw meat and reported that among the antibiotics, gentamycin was the most

effective with 98.9% efficacy followed by ofloxacin (91.8%), norfloxacin (76.5%), ciprofloxacin (76.2%), kanamycin (72.6%), cotrimoxazole (61.6%), tetracycline (59.4%), chloramphenicol (53.4%), whereas cephalexin (47.1%), ampicillin (46.6%), erythromycin (11.4%), carbenicillin (8.2%), methicillin (7.1%) cloxacillin (6.8%), and penicillin G (6.0%) were found to be resistance Murray *et al*. (2001) found the mean total viable count, mean yeast count and mean Enterobacteriaceae counts as log 2.75 ± 0.64, 0.46 ± 0.5 and 0.04 ± 0.30/g respectively in beef.

Siriken (2002) studied on microbiological quality of ground beef in Aydin and Afyon Provinces, Turkey. He found that out of 70 ground beef sample, 79 % of the samples contained $>10^5$ aerobe mesophile plate count, 44 % $>10^2$ cfu/g *Pseudomonas*, 47 % $>10^3$ cfu/g enterobacteriaceae, 65 % $>10^3$ cfu/g enterococci, 42 % $>10^3$ cfu/g Micrococci/Staphylococci and 64 % contained at >1100 MPN/g coliforms. Coagulase positive Staphylococci, *E. coli,* and *Salmonella* spp were detected in 21.4 %, 30 %, 10 % of the samples respectively.

Sachindra *et al.* (2003) studied on the microbial levels of buffalo sausage during preparation and storage at 4±1 ^{0}C and found microbial contamination may be added or reduced at different stages of processing of buffalo sausage. Microbial levels of raw minced meat are total plate counts (logcfu/g) 5.41±0.25, coliforms (MPN/g) 23.2, *S. aureus* (logcfu/g) 1.57±0.11, yeast and molds (logcfu/g) 2.29±0.07 and lactic acid bacteria (logcfu/g) 0.60±0.20 and raw sausage stuffed in casing are TPC (logcfu/g) 5.10±0.35, coliforms (MPN/g) 98, *S. aureus* (logcfu/g) 1.48±0.03, yeast and molds (logcfu/g) 2.50±0.06 and Lactic acid bacteria (logcfu/g) 0.70±0.01.

Sumner *et al.* (2003) examined 159 chilled beef carcasses processed from four abattoirs and 13 very small plants and reported 1.82 log AVC/cm2 and 1.72 log AVC/cm^2 aerobic viable counts at abattoirs and very small establishments

respectively. The prevalence of *E. coli* from 200-cm^2 areas sampled was 28.4% at abattoirs and 4.7% at very small establishments.

Elami M and Yamen H (2005) examined 100 sample of raw ground beef (n=50) and raw meat ball (n=50) and found the mean counts (CFU/g) of total aerobic mesophiles, and coliform as $4.3x\ 10^6$, and $1.7\ x\ 10^4$ respectively in ground beef sample. Of the 50 ground beef sample, 12 (24%) sample contain *Salmonella* spp. and 1 (2%) sample contains *E. coli*.

Elmali *et al.* (2005) carried out a seven month survey for the detection of *E. coli* O157:H7 from ground beef samples in the Markets of Turkey and observed that the incidence of EHEC serotypes were only in April. Of the 126 ground beef samples, only one ground beef sample was positive for *E. coli* O157:H7, having a prevalence of 0.79 %. And five samples were found positive for *E. coli* O157 serotype, having a prevalence of 3.96 %. Antibiotic resistance patterns of *E. coli* O157 *and E. coli* H7:O157 were determined. *E. coli* O157 and *E. coli* O157:H7 serotypes were resistant to Chloramphenicol, Streptomycin, Oxytetracycline, Tetracycline, Trimethoprim.

Joshi *et al.* (2005) studied on the prevalence of *Salmonella* in meat in Kathmandu. A total of 123 sample including 55 chickens, 37 buffaloes, and 31 chevon were collected. *Salmonella* was isolated from 14(11.38%) sample. Varity wise 14.5% chicken sample, 13.15% buffaloes and 3.2% chevons sample were found to be positive for *Salmonella* present and 80% of chicken, 89% of buff meat and 70% of chevon were found to be positive for the presence of coli form especially *E. coli*.

Nazmul *et al*. (2006) carried out the research work on the microbiological analysis of buffalo meat. They found that among 60 meat sample, 64% were unsafe and unhygienic for consumption from public health point of view as compared with the standard permissible limit specified by BIS (IS2537:1995).

Staphylococcus species was found in 24% of sample and only few numbers of *Salmonella* was detected.

Phillips *et al.* (2006) examined 1,155 beef carcasses and 1,082 frozen boneless beef and found that a mean aerobic plate count (at 25°C) of beef carcass was 1.3 log CFU/cm^2. *Escherichia coli* in 8.0% of the beef carcasses, with a mean count of 0.8 log CFU/cm^2. *E. coli* O157:H7 was isolated from 1 of 1,143 carcasses. No *Salmonella* and *Campylobacter* spp were isolated from carcasses. Coagulase-positive staphylococci were found in 28.7% of beef carcasses at mean count of 0.3 log CFU/cm^2.

Selvan *et al.* (2007) examined six sample of beef product(beef minced and beef sausages) and found mean value (log cfu/g) of total viable count, psychrotrphic count, anaerobic count, coliform count, streptococcal count, and staphylococcal count as 4.78±0.19, 3.33± 0.01, 3.2±0.02, 3.68±0.19, 5.16±0.02, and 2.07±0.38 respectively. No *Salmonella* was isolated from beef sample.

Podpecan *et al.* (2007) studied on the sources of contamination of ground meat for production of meat products with bacteria *Staphylococcus* and found that the major source of contamination of ground meat are the hands of workers (contamination of workers' hands with bacteria *S. aureus* was 58.33 % of the specimen taken after the handling of five beef carcasses) and the contaminated surface of beef carcass (44.4 % of smears of surface of beef carcasses was contaminated with bacteria *S. aureus*). The bacteria *S. aureus* was isolated on the thorax in 78 % (39/50) of the specimens, 62 % (31/50) on the front legs, 58 % (29/50) on the abdomen wall, 14 % (7/50) on the thigh and 10 % (5/50) on the neck.

Hao Van *et al.* (2007) examined 180 samples of meat, comprising beef (n = 50), chicken/poultry (n = 30), pork (n = 50), and shellfish (n = 50) purchased from 14 markets and 4 supermarkets around Ho Chi Minh City and found 32 (64%) of

pork samples, 31 (62%) of beef samples, 16 (53.3%) of chicken samples and 18% of shellfish sample were contaminated with *Salmonella* spp. Ninety-one *Salmonella* isolates recovered from food samples were tested for antibiotic resistance against 15 antibiotics:- AMP, amoxicillin augmentin, cephalothin, CHL, ciprofloxacin, enrofloxacin, TET, gentamicin, kanamycin, nalidixic acid, norfloxacin; sulfafurazole, STR and trimethoprim. It was observed that approximately half (50.5%) of the isolates were resistant to at least one antibiotic. Multiresistant *Salmonella* isolates were observed in all food types. The rates were 34.4%, 27.8%, 11.1%, and 6.3% in pork, chicken, shellfish, and beef isolates, respectively.

Cohen *et al.* (2008) examined 250 samples of raw ground beef (n=150) and fresh sausages (n=100) collected from butchers, supermarkets and fast food shops in Casablanca, Morocco. They found the mean log cfu/gm of aerobic plate count and fecal coliform count of raw beef meat as 7.3±0.3, 7.3±0.3 and 7.6±0.3; and 3.5±0.4, 3.3±0.4, and 3.8±0.4 and that of the sausages as 7.2±0.4, 7.1±0.4, and 7.3±0.4; and 3.7±0.5, 3.6±0.5 and 3.2±0.5 from butchner, supermarket and fast food shop respectively. *S. aureus* was isolated from 25 sample of raw ground beef and 18 sample of fresh sausages, *Salmonella, Cl. perfringens* and *L. monocytogenes* from 3, 29 and 3 sample of raw ground beef and 4, 18 and 5 sample of fresh sausages respectively.

Soyiri *et al.* (2008) examined 128 fresh beef samples and reported aerobic mesophiles, *S. aureus, B. cereus, Cl. perfringens* and *E. coli* count at level of 189-23000 cfu/g, 22-59 cfu/g, 17-41 cfu/g, 21-48 cfu/g 31-2200 cfu/g respectively. *E. coli* were found to be the predominant microbial contaminant in the samples examined.

Mukhopadhya *et al.* (2009) studied on microbial quality of fresh chevon and beef in retail retail outlet of pondicherry and found the mean aerobic plate count, mean coliform count and mean yeast and mould count for chevon samples as log10

7.76, log10 6.40 and log10 6.90 cfu /g respectively and for beef sample as log10 6.66, log 10 5.84 and log 10 6.25 respectively. Out of the 23 chevon sample tested, *S. aureus* was isolated from 5 sample and corneybacterium from 2 chevon and 1 beef sample.

Bosilevac *et al.* (2009) carried out a survey on prevalence and characterization of Salmonellae in commercial ground beef and reported *Salmonella* prevalence of 4.2% identified by isolating 172 salmonellae in 4,136 ground beef samples collected from seven regions of the United States All *Salmonella* isolates were serotyped and their antibiotic susceptibilities determined and analyzed by pulsed-field gel electrophoresis (PFGE). The most common serotypes identified were *Salmonella enterica* serotypes Montevideo, Anatum, Muenster, and Mbandaka. The prevalence of multidrug-resistant (MDR) *Salmonella* was 0.6%. The most common MDR serotypes were *Salmonella enterica* serotypes Dublin, Reading, and Typhimurium.

Clarence *et al.* (2009) studied on bacteriological quality of ready to eat food (Meat pie). Eight triplicate samples of meat pie were collected from standard eatery and local kiosk in Benin City. They found the mean microbial load on fresh meat pie from the standard eatery ranged from $3x10^3 – 5x10^3$cfu/g and from the local kiosk ranged between $7x10^3$-$2.8x10^4$ cfu/g. Six genera of the bacteria were isolated and identified as *Staphylococcus, E. coli, Klebsiella, Pseudomonas, Bacillus* and *Enterococcus.*

Salihu *et al.* (2009) examined 216 samples of fried ground beefs purchased from different retail outlets located in and around Sokoto metropolis and found the prevalence of aerobic mesophiles, feacal coliforms, *E. coli* and *S. aureus* as 100, 49.5, 36.6 and 69.9% respectively. The counts of aerobic mesophiles, feacal coliforms, *E. coli* and *S. aureus* ranged between 6.70 $x10^8$ and $9.30x10^9$ CFU/g, 10^3 and 10^5 CFU/g, 10^2 and 10^5CFU/g and 10^5 and 10^7 CFU/g respectively.

Enabulele and Uraih (2009) examined 72 samples each of fresh meat from abattoir and open traditional market and "ready to eat" grilled meat (suya) and found the overall prevalence rate *E. coli* and *E. coli* 0157: H7 were 85.65 and 4.63% respectively, fresh meat samples from abattoir and traditional open market each, recording 100% *E. coli* prevalence. Fresh meat from abattoir had the highest *E. coli* 0157: H7 prevalence (6.94%) while market samples had the lowest (2.78%).

Iroha *et al.* (2010) examined bacteria contamination of raw meat and found that out of 300 meat sample including beef (n=100), chicken (n=100), chevon (n=100), 79 (29.3%) were found to be contaminated with different kind of microorganisms. In the sample most frequently *E. coli* (8%), *K. pneumoniae* (5.3%), *S.* Typhi (5%), *S. dysenteriae* (2.6%), *P. aeruginosa* (2.0%), *B. cereus* (2.0%) and *S. aureus* (1.3%) were observed. Sixteen different antibiotic discs were used to test susceptibility patterns of the isolated organisms. The susceptibility results of bacterial isolates showed that they are highly resistance to all the antibiotics tested. Gram-negative organisms are more resistant than the Gram-positives organism.

Omoruyi *et al.* (2011) examined bacteriological quality of beef-contact surfaces, air microflora and wastewaters from major abattoirs and reveal total heterotrophic counts from air flora ranged from 14.50×10^6 to 42.50×10^6cfu. Beef-contact surface ranged from 26.50×10^6 to 592.50×10^6cfu while total colony counts obtained from wastewaters from both government and private abattoirs ranged from 140.00×10^6 to 1206.75×10^6cfu/ml. The total coliform counts also ranged from 14.25×10^3 to 33.75×10^3 for air flora and 76.00×10^3 to 195.00×10^3cfu/ml for wastewaters. Eight bacterial isolates were isolated and they included; *E. coli, S. aureus, Staphylococcus* spp, *Citrobacter* spp, *Alcaligenes paradoxus, Klebsiella* spp and *Enterococcus faecalis* with varying percentage of frequency across the sampling points.

Koffi-Novry *et al.* (2011) studied the bacteriological quality of the beef meat produced for retail sale in Cote d' ivore. The bacterial load was assessed on sample collected at regular interval from 6:00 AM to 6:00 PM and found mean counts (log 10 CFU/g) of total aerobic microorganisms, faecal coliforms, *S. aureus, Pseudomonas* as 4.93, 1.83, 1.53, and 1.29 at 6:00AM and 8.1, 4.73, 2.43, and 2.79 at 6:00 PM respectively. Out of 300 sample collected from 60 beef carcasses, *Salmonella* was found in 27% of the meat sample.

Ali *et al.* (2010) examined 340 sample (250 raw meat sample and 90 surface swabs from meat processing equipment and the surrounding environment) and found the total aerobes was counted at ranged between 10^8-10^{10} CFU/g and 84% of sample were contaminated with bacterial species. 342 potential pathogenic bacterial isolates were isolated from meat sample. The most frequently isolated bacterial pathogen from meat sample were *E. coli* 120(35%), L*isteria* 14(4%), *Klebsiella* 27(8%), *Enterobacter* spp 51(15%), *Staphylococcus* 24(7%), *Salmonella* Enteritis 24(7%), *Shigella* 27(8%) and *Brucella* 4(1%).

Atwa and Nahla Abou EI-Roos (2011) studied the SIncidence of *Clostridium perfringens* in Meat Products at Some Egyptian Governorates and show the prevalence of *Cl. perfringens* from ready to cook meat products was 48.8% with the incidence of 28%, and 68 % from minced meat, and beef sausage respectively. The incidence of toxigenic and non toxigenic strains of *Cl. perfringens* was 89.6% and 10.4%, respectively. The toxigenic strains of *Cl. perfringens* type A was the most predominant one (46.8%), while type D and mixed types have the incidence of 19.5% and 23.3%, respectively. The mean *Cl. perfringens* counts of minced meat, and beef sausage were 1.2x 10^3 and 1.2x 10^3cfu /g respectively.

Al-Mutairi (2011) studied on the incidence of enterobacteriaceae causing food poisoning in some meat products collected from different supermarkets and shops in Giza governorate, Egypt. He found the mean aerobic plate, enterobacteriaceae, the coliforms (MPN) count values of sausage as 24.3×10^4 CFU/g, 5×10^4 CFU/g

and 3×10^2 CFU/g, respectively. Out of 25 sausages sample, *E. coli*, *Salmonella* spp, *Klebsiella* spp and *Proteus* spp were isolated from 3(12%), 2(8%), 1(4%) and 2(8%) respectively.

Victoria and Tajudeen O (2011) examined the antibiotic resistance of *E. coli* and *Salmonella* isolated from retail meat. Among the antibiotics disc used, *E. coli* was sensitive to cefuroxime, ciprofloxacin, norfloxacin and gentamicin, but was resistance to tetracycline, nitrofurantoin, ofloxacin, amoxillin, ampicillin and chloramphenicol. *Salmonella* spp. was highly sensitive to gentamicin and amoxillin but was resistance to tetracycline, ciprofloxacin, ampicillin, ofloxacin and norfloxacin.

CHAPTER – III
MATERIALS AND METHODS

3.1 Methodology

Standard microbiological methodology for sample collection and isolation was used to complete this study in the Central Department of Microbiology, Tribhuvan University, Kirtipur.

The study was done between the November 2010 to May 2011 at nine different sites Byasi, Kamalbinayak, Chayamasingh, Sukuldhoka, Suryamadhi, Bholachee, Barahisthan, Thimi, and Lokanthali to identify the total bacterial count, total coliform count and to identify the isolated organism and to determine the antibiotics susceptibility pattern of the isolates. In total 75 samples; 45 raw buffaloes meat and 30 sausages sample; were studied.

3.2 Sampling sites

Total 9 sampling site were chosen randomly in Bhaktapur district in purposes to get the homogenous sample for the study. The sampling sites at different location were: Byasi, Kamalbinayak, Chayamasingh, Sukuldhoka, Suryamadhi, Bholachee, Barahisthan, Thimi, and Lokanthali. In order to get the homogenous sample 45 raw buffaloes meat sample 5 from each sites and 30 sausages sample were randomly collected for the study of microbiological quality of meat and sausages.

3.3 Sample collection

It is utmost importance that samples of foods collected for microbiological analysis accurately reflect the microbiological conditions at the time of sampling. Therefore sampling was carried out using standard microbiological method. Sample were collected aseptically in a clean plastic bag and immediately carried to the Microbiology Laboratory of Central Department, Tribhuvan University,

Kirtipur. Examination usually started on that day or in some cases the sample were kept in refrigerator and tested the next day.

3.4 Processing of the sample

3.4.1 Enumeration of aerobic mesophilic count and coliform count

3.4.1.1 Meat sample preparation

I. Grinding of meat: Twenty five gram of the sample was aseptically transferred into a sterile mortar and grinded by sterile pestle and sterile knife added to 245 ml of sterilized buffered peptone water. This sample homogenate was labeled as 10^{-1} dilution.

II. Serial dilution of homogenate: The homogenate was mixed well by shaking. 1ml of the homogenate was pipetted out into a tube containing 9 ml of buffered peptone water and carefully mixed and labeled as 10^{-2} dilution. Similarly, the dilution was carried out upto the 10^{-7} dilution and labeled as 10^{-3}, 10^{-4} and so on to 10^{-7} dilution respectively.

III. Pour plating

A. Isolation and Enumeration of aerobic mesophilic bacteria

One ml of homogenate and dilution of the homogenate were pippeted out and put into each of the sterile appropriately marked plates. Sterilized total plate count agar (TPCA), cooled to 45^0 C, and was poured into each petridish within 15 minutes of the time of original dilution. The sample dilution and agar medium were mixed thoroughly and uniformly and allowed to solidify. Then the Petri plates were incubated at 37 ^{o}C for 24 hrs.

B. Isolation and Enumeration of coliform bacteria

One ml of homogenate and dilution of the homogenate were pippeted out and kept into each of the sterile appropriately marked plates. Sterilized violet red bile

agar (VRBA), cooled to 45^0C, and was poured into each Petri plate within 15 minutes of the time of original dilution. The sample dilution and agar medium were mixed thoroughly and uniformly and allowed to solidify. Then the Petri plates were incubated at 37 ^{o}C for 24 hrs.

IV. Counting of the colonies

The Petri plates containing 30-300 colonies after 24 hours incubation were counted and calculation was done as:

- When the plates examined contained no colonies, the result was expressed as zero bacteria per gm/ml.
- When the plates (dilution 1 in 10) contain less than 30 colonies and no other plates of the sample contain colonies then it was counted and the result was expressed as the (number of colonies) CFU of the bacteria per gm/ ml.
- When the colonies were more than 30, in more than one plate, the colonies in the plate were counted and the average was counted, retaining only two significant digits and multiply by the inversed of the corresponding dilutions to obtain the number of the bacteria per gm/ml.

3.4.2 Isolation of *Salmonella* and *Shigella* species

I. Enrichment: 5 gram of the grinded sample was aseptically transferred to the sterile conical flask containing 45ml of Selenite-F broth, and incubated at 37^0C for 24 hours for enrichment of *Salmonella* and *Shigella*.

II. Plating out: After enrichment, a loopful of the enriched culture from Selenite-F broth was streaked on Xylose-Lysine- Deoxycholate (XLD) Agar and incubated at 37^0C for 24 hours. Growth observed as red colonies with or without blacked centre on XLD was picked up.

3.4.3 Isolation of *Staphylococus* species

The homogenate was streaked on Mannitol Salt Agar (MSA) and incubated at 37^0C for 24 hours. The mannitol fermenting colonies i.e golden yellow colonies were picked up and sub culture on Nutrient agar plate in order to get pure colonies and incubated at 37^0C for 24 hours

3.4.4 Isolation of the organisms

The homogenate was streaked on Eosin Methylene Blue (EMB) agar, and Mac-Conkey agar (MA) and incubated at 37^0C for 24 hours. The organism with typical colonial character in VRBA (used for enumeration of coliform) were picked and sub culture on Nutrient Agar (NA) plate and Mac-Conkey agar (MA) plate and incubated at 37^0C for 24 hours.

3.5 Identification of the organisms

After obtaining the pure culture, the organism was identified by using standard microbiological techniques as described in BERGEY'S MANUAL OF SYTEMATIC BACTERIOLOGY-1986 which involves morphological appearance of the colonies, Gram Staining, and Biochemical test (Monica Cheesbrough, 1984).

Biochemical tests used for identification of isolated bacteria:

Following biochemical tests were performed to identify the isolated organisms.

Table 1 Biochemical Tests performed for Identification of Enteric Bacteria

S.N.	TEST	BIOCHEMICAL MEDIA
1.	Catalase	3% H_2O_2
2.	Oxidase	10% Tetramethyl - p- phenyle diamine dihydrochloride.
3.	Indole Production	Sulfide- Indole- Motility medium (SIM)
4.	Methyl Red test	Glucose phosphate peptone water or MR-VP

		medium.
5.	Voges- Proskauer test	Glucose phosphate peptone water or MR-VP medium.
6.	Citrate utilization test	Simmon's citrate agar.
7.	Fermentation of glucose, lactose and sucrose, H_2S and gas production	Triple sugar Iron Agar (TSIA)
8.	Aerobic or anaerobic utilization of carbohydrate	Hugh and Leifson Medium.
9.	Urease Production	Urea base agar.

For the identification of gram positive organisms, the following test was carried out: Gram staining, catalase, oxidase test, OF test, coagulase test.

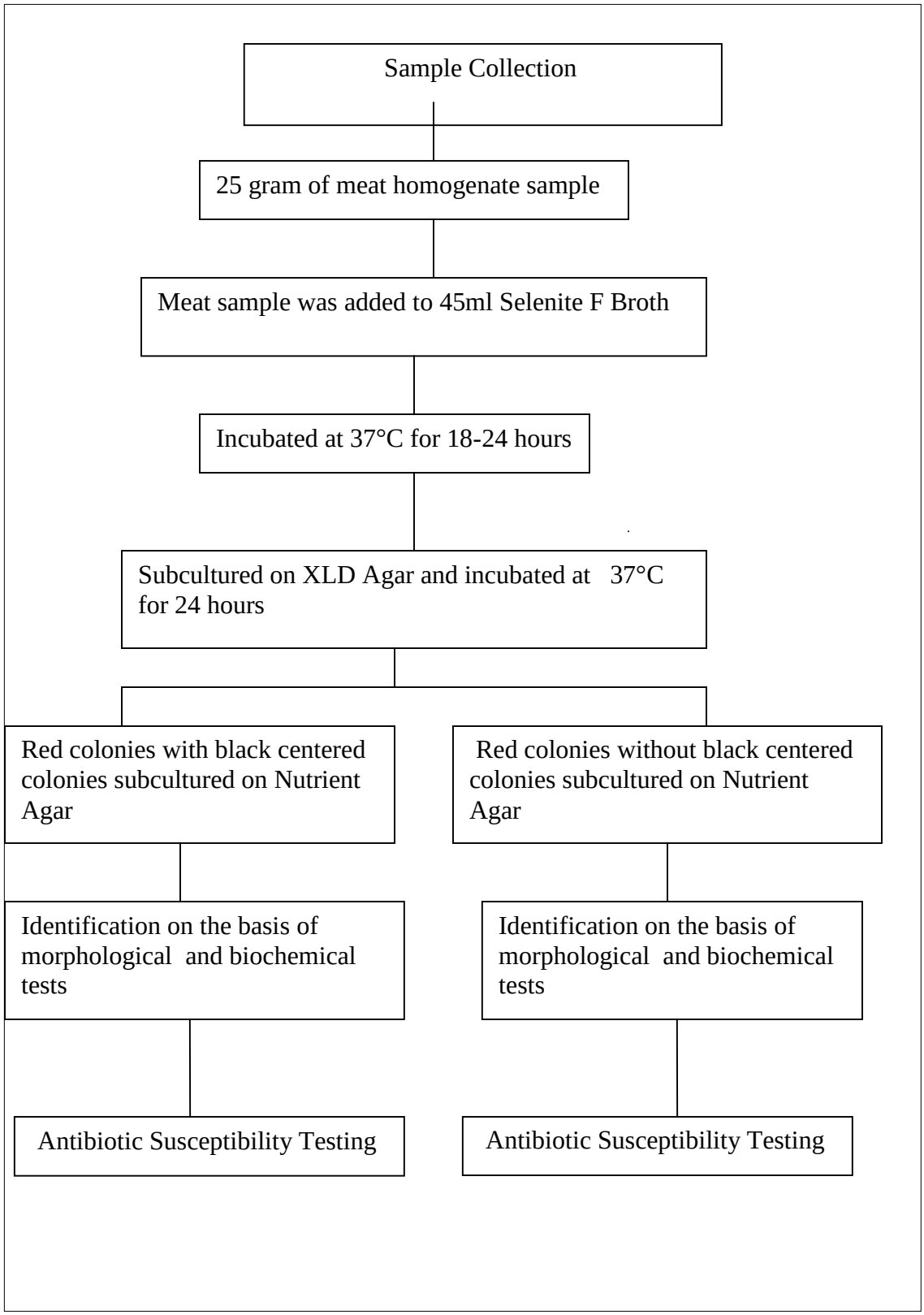
3.6 Study of antibiotic susceptibility of isolated organisms

Antibiotic susceptibility test of isolated organism was assayed using CLSI recommended a modified Kirby-Bauer disk diffusion method (Bauer et al., 1966). Microorganisms were grown at 37^{o}C in 5ml of nutrient broth for about 4 hours using pure cultures as inoculums. The turbidity developed was compared to 0.5% Mc Farland Standard tube. A sterile cotton swab was dipped into the properly prepared inoculums and firmly rotated against the upper inside wall of the tube to remove excess inoculums, and then swabbed over the entire surface of the dried Muller-Hinton agar plate. During swabbing the plate was rubbed with the swab three times turning the plate 60^{o}C between each streaking to achieve a lawn of confluent bacterial growth. The plate was kept at room temperature for 5 to 10 minutes, but no longer than 15 minutes to dry the inoculums. Antibiotics discs from their respective vials were carefully placed in the plate with the help of a flamed forceps, at equal distance and sufficiently separated from each other to avoid the overlapping of the inhibition. The antibiotics discs were gently pressed with the forceps to make complete contact with the surface of the medium. The

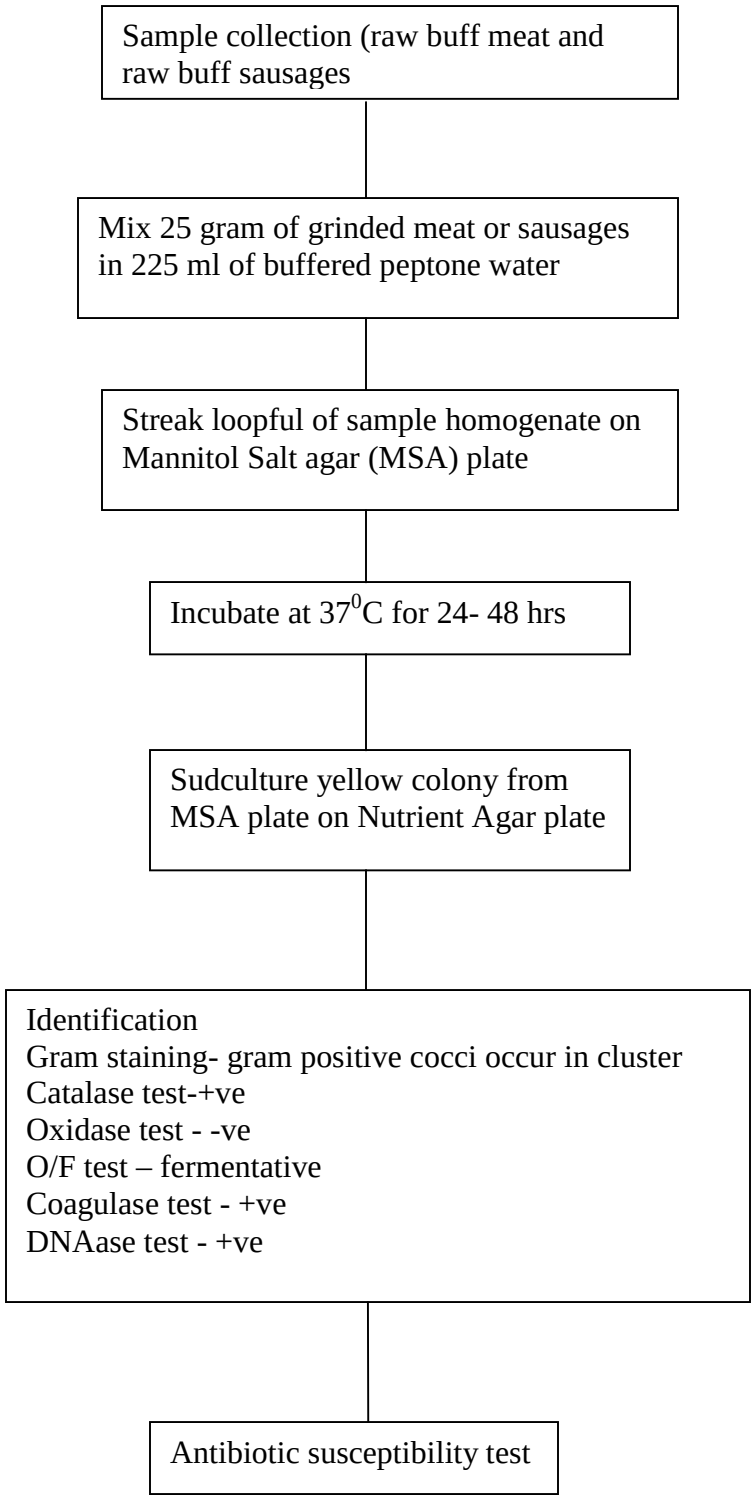
plate was allowed to stand at room temperature for 30 minutes for pre diffusion and then incubated at 37^0C for 24 hrs. The diameter of the zone of inhibition around each antibiotics disc was measured after 24 hrs of incubation. Organisms were classified as sensitive or resistant to an antibiotic according to the diameter of the inhibition zone surrounding each antibiotic disc as listed by manufacturer.

A wide range of antibiotics namely tetracycline, amikacin, chloramphenicol, nalidixic acid, ofloxacin, and cotrimoxazole were included for antibiotics susceptibility test.

Flow chart for Isolation of *Salmonella* and *Shigella*



Flow chart for isolation and identification of *Staphylococcus aureus*



CHAPTER-IV
RESULTS

4.1 Microbiological study of buff meat

Buff meat was sampled from 9 different sites: - Lokanthali, Thimi, Sukuldhoka, Suryamadhi, Barahisthan, Chayamasingh, Kamalbinayak, Byasi and Bholachhe. Althogether 45 buff meat sample; 5 sample from each location were taken. The samples were processed for total plate count, total coliform count and identification of the isolates were carried out following the standard microbiological processes.

It was found that the highest total plate count were found to be from the sample of Kamalbinayak with mean value $1.4x10^7$cfu/gm and the lowest total plate count from Barahisthan with mean value $1.9x10^6$cfu/gm whereas the highest coliform count were found to be from the sample of chayamasingh with mean value $1.3x10^6$cfu/gm and the lowest aerobic count from suryamadhi with mean value $6.3x10^4$cfu/gm.

Table 1: Total plate count and coliform count of different buff meat samples.

S.N	Location	Sample code	Total plate count (cfu/gm)	Average count (cfu/gm)	Total coliform count (cfu/gm)	Average count (cfu/gm)
1	Lokanthali	LM1	$1.6x10^7$		$1.4x10^5$	
		LM2	$1.1x10^7$		$1.2x10^5$	
		LM3	$4.6x10^5$	$6.2x10^6$	$3.8x10^3$	$1.0x10^5$
		LM4	$1.3x10^6$		$2.2x10^5$	
		LM5	$3.3x10^5$		$3.1x10^4$	
2	Thimi	TM1	$2.8x10^6$		$2.4x10^4$	
		TM2	$1.9x10^6$			
		TM3	$2.5x10^6$	$4.5x10^6$	$3.1x10^4$	$6.7x10^4$
		TM4	$1.1x10^7$		$1.7x10^5$	
		TM5	$4.1x10^6$		$1.1x10^5$	
3	Sukuldhoka	SuM1	$1.0x10^7$		$2.1x10^5$	
		SuM2	$2.3x10^7$		$1.4x10^5$	$1.1x10^5$
		SuM3	$2.6x10^6$	$1.1x10^7$	$2.5x10^4$	

		SuM4	$1.6x10^7$		$1.2x10^5$	
		SuM5	$3.7x10^6$		$3.2x10^4$	
4	Suryamadhi	SM1	$1.2x10^6$		$1.3x10^4$	
		SM2	$2.9x10^6$		$2.1x10^4$	
		SM3	$2.1x10^7$	$5.7x10^6$	$2.0x10^5$	$6.3x10^4$
		SM4	$1.9x10^6$			
		SM5	$1.7x10^6$		$1.7x10^4$	
5	Barahisthan	BM1	$2.6x10^6$		$1.6x10^4$	
		BM2	$3.7x10^6$		$2.3x10^5$	
		BM3	$1.4x10^6$	$1.9x10^6$		$6.5x10^4$
		BM4	$6.0x10^5$		$2.1x10^3$	
		BM5	$1.5x10^6$		$1.3x10^4$	
6	Chayamasingh	CM1	$3.9x10^6$		$1.8x10^5$	
		CM2	$3.7x10^6$		$1.6x10^5$	
		CM3	$1.2x10^7$	$1.0x10^7$	$3.1x10^6$	$1.3x10^6$
		CM4	$4.6x10^6$		$2.4x10^5$	
		CM5	$2.7x10^7$		$2.9x10^6$	
7	Kamalbinayak	KM1	$2.1x10^7$		$2.4x10^6$	
		KM2	$1.7x10^7$		$2.5x10^6$	
		KM3	$3.1x10^6$	$1.4x10^7$	$1.2x10^5$	$1.2x10^6$
		KM4	$2.6x10^7$		$8x10^5$	
		KM5	$3.2x10^6$		$1.4x10^5$	
8	Byasi	ByM1	$2.3x10^6$		$4.1x10^5$	
		ByM2	$1.2x10^7$		$2.3x10^6$	
		ByM3	$1.7x10^6$	$6.4x10^6$	$4.8x10^4$	$1.0x10^6$
		ByM4	$3.1x10^6$		$1.0x10^6$	
		ByM5	$1.3x10^7$		$8.5x10^5$	
9	Bholachee	BoM1	$1.2x10^6$		$1.7x10^5$	
		BoM2	$1.5x10^6$			
		BoM3	$2.5x10^6$	$3.6x10^6$	$3.0x10^4$	$5.5x10^5$
		BoM4	$3.1x10^6$		$1.1x10^5$	
		BoM5	$1.0x10^7$		$1.9x10^6$	

Bacteriological profile of raw buff meat

In this study, a total of 100 bacteria of 8 different genera were isolated. The organisms identified include *Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter spp, Citrobacter diversus, Shigella* spp, *Salmonella* spp, *Salmonella* Typhi, *Proteus mirabilis, Proteus vulgaris* and *Staphylococcus aureus*. Percentage of *E .coli* (34.0%) was found to be highest followed by *K. oxytoca* (12.0%), *C. diversus* and *Enterobacter* spp (10.0%) *C.*

freundii and *S. aureus* (7.0%), *P. vulgaris* (5.0%), *Salmonella* Typhi and *K. pneumoniae* (4.0%) and *P. mirabilis, Salmonella* spp and *Shigella* spp (2.0%).

Table 2: Bacterial isolates from different buff meat sample

S.N	Location	Sample code	Organism identified
1	Lokanthali	LM1	*E. coli, Klebsiella oxytoca, Staphylococcus aureus*
		LM2	*E. coli, Enterobacter* spp
		LM3	*E. coli, Citrobacter diversus*
		LM4	*E. coli, Klebsiella oxytoca*
		LM5	*E. coli, Proteus vulgaris*
2	Thimi	TM1	*E. coli, Citrobacter freundii, Salmonella* spp
		TM3	*E. coli, Proteus mirabilis, Citrobacter freundii*
		TM4	*E. coli, Enterobacter* spp
		TM5	*E. coli, Staphylococcus aureus*
3	Sukuldhoka	SuM1	*E. coli, Klebsiella oxytoca, Citrobacter diversus*
		SuM2	*E .coli, Salmonella* Typhi
		SuM3	*E. coli, , Citrobacter. freundii*
		SuM4	*E. coli, Klebsiella pneumoniae*
		SuM5	*Citrobacter diversus, Staphylococcus aureus*
4	Suryamadhi	SM1	*E. coli, Shigella* spp, *Enterobacter* spp
		SM2	*Klebsiella pneumoniae, Enterobacter spp, Staphylococcus aureus*
		SM3	*E. coli, Klebsiella oxytoca, Citrobacter diversus*
		SM5	*Citrobacter diversus, Proteus vulgaris*
5	Barahisthan	BM1	*E. coli, Klebsiella oxytoca, Salmonella* Typhi
		BM2	*E. coli, Citrobacter diversus, Proteus vulgaris*
		BM4	*E. coli, Klebsiella oxytoca, Citrobacter diversus*
		BM5	*E. coli, Klebsiella pneumoniae,, Enterobacter* spp
6	Chayamasingh	CM1	*E. coli, Klebsiella oxytoca, Proteus mirabilis*
		CM2	*E .coli, Citrobacter diversus, Enterobacter* spp
		CM3	*Klebsiella pneumoniae,Staphylococcus aureus*
		CM4	*E. coli, Citrobacter freundii*
		CM5	*Klebsiella oxytoca*
7	Kamalbinayak	KM1	*E. coli, Klebsiella oxytoca, Citrobacter diversus, Staphylococcus aureus*
		KM2	*E. coli, Klebsiella oxytoca, Citrobacter diversus*
		KM3	*E. coli, Salmonella* spp
		KM4	*Enterobacter* spp, *Citrobacter freundii*
		KM5	*E. coli, Enterobacter* spp, *Protreus vulgaris*
8	Byasi	ByM1	*E. coli, Klebsiella oxytoca, Proteus mirabilis*
		ByM2	*E. coli , Salmonella* Typhi
		ByM3	*E. coli, Citrobacter freundii*
		ByM4	*E. coli, Proteus vulgaris*
		ByM5	*E. coli, Klebsiella oxytoca, Staphylococcus aureus*
9	Bholachhe	BoM1	*E. coli, Enterobacter* spp, *Salmonella* Typhi
		BoM3	*Enterobacter* spp, *Shigella* spp

		BoM4	*E. coli, Citrobacter freundii*
		BoM5	*E. coli*

4.2 Microbiological study of raw buff sausages

Sausages sample were purchased from the minimarket (n=5), butchner (n=5) and fast food shop (n=20). The samples were studied for total plate count, total coliform count and identification of the organism.

It was found that the highest total plate count were found to be from the sample collected from butchner with mean value $8.3x10^5$cfu/gm and the lowest total plate count from sample collected from minimarket with mean value $1.5x10^5$cfu/gm where as the highest coliform count were found to be from the sample collected from fast food shop with mean value $3.5x10^4$cfu/gm and the lowest coliform count from sample collected from minimarket with mean value $1.3x10^3$cfu/gm.

Table 3: Total Plate count and coliform count of different buff sausages sample

S.N	Sample	Sample code	Total plate count (cfu/gm)	Average count	Total coliform count (cfu/gm)	Average count
1	Buff sausages (collected from market)	S1	$1.5x10^5$		$1.2x10^3$	
		S2	$3.5x10^5$		$1.6x10^3$	
		S3	$2.4x10^4$	$1.5x10^5$	$1.2x10^3$	$1.3x10^3$
		S4	$1.2x10^4$			
		S5	$2.4x10^5$			
2	Buff sausages (collected from butchner)	S6	$2.9x10^5$		$2.4x10^4$	
		S7	$1.0x10^6$		$4.9x10^4$	
		S8	$4.9x10^5$	$8.3x10^5$		$2.3x10^4$
		S9	$1.6x10^6$		$1.3x10^4$	
		S10	$7.9x10^5$		$5.2x10^3$	
3	Buff sausages (collected from fast food shop)	S11	$5.2x10^5$			

		S12	$9.6x10^5$		$8.1x10^3$	
		S13	$8.1x10^4$		$3.3x10^4$	
		S14	$1.6x10^6$		$1.8x10^5$	
		S15	$3.8x10^5$			
		S16	$1.9x10^6$		$2.9x10^4$	
		S17	$2.1x10^5$		$6.7x10^3$	
		S18	$1.4x10^6$		$4.3x10^4$	
		S19	$6.1x10^5$		$1.7x10^4$	
		S20	$2.3x10^5$	$7.5x10^5$		$3.5x10^4$
		S21	$1.0x10^6$			
		S22	$3.4x10^5$		$2.7x10^4$	
		S23	$4.3x10^5$			
		S24	$6.4x10^5$		$1.5x10^5$	
		S25	$1.2x10^6$		$2.1x10^4$	
		S26	$3.2x10^5$			
		S27	$2.7x10^5$			
		S28	$5.3x10^5$		$4.2x10^4$	
		S29	$4.8x10^5$			
		S30	$1.8x10^6$		$3.9x10^3$	

Bacteriological profile of raw buff sausages

In this study, a total of 46 bacteria of 8 different genera were isolated. The organisms identified include *Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae, Klebsiella oxytoca*, *Enterobacter spp, Citrobacter diversus, Shigella* spp, *Salmonella* Typhi, *Proteus mirabilis* and *Staphylococcus aureus*. Percentage of *E. coli* (31.9%) was found to be highest followed by *K. pneumonia (14.8) C. freundii* and *S. aureus* (12.7%), *C. diversus* (8.5%), *Enterobacter* spp and *K. oxytoca* (6.3%) *Salmonella* Typhi, *Shigella* spp and *P. mirabilis* (2.1%)

Table 4: Bacteriological profile of raw buff sausages

S.N	Sampling site	Sample code	Organism isolated
1	Market	S1	*E. coli, Klebsiella pneumoniae*
		S2	*E. coli, Citrobacter freundii*
		S3	*Klebsiella pneumoniae*
2	Butchner	S6	*E. coli, Klebsiella pneumoniae, Staphylococcus aureus*
		S7	*Citrobacter diversus, Enterobacter spp*
		S9	*E. coli, Proteus mirabilis, Staphylococcus aureus*
		S10	*E. coli*
3	Fast food shop	S12	*E. coli, Klebsiella pneumoniae*

		S13	*E. coli, Citrobacter freundii*
		S14	*E. coli, Enterobacter spp*
		S16	*E. coli, Klebsiella oxytoca, Citrobacter freundii*
		S17	*Klebsiella pneumoniae, Citrobacter freundii Staphylococcus aureus*
		S18	*Salmonella* Typhi, *Staphylococcus aureus, Citrobacter diversus*
		S 19	*E. coli, Klebsiella oxytoca, Shigella spp*
		S22	*E. coli, Klebsiella oxytoca, Citrobacter freundii*
		S24	*E. coli, Enterobacter spp, Citrobacter freundii*
		S25	*E. coli, Citrobacter diversus, Staphylococcus aureus*
		S28	*E. coli, Klebsiella pneumoniae, Staphylococcus aureus*
		S30	*E. coli, Klebsiella pneumoniae, Citrobacter diversus*

Table 5: Frequency of occurrence of isolates from the meat sample

Bacteria isolates	Buff meat	Buff sausages	% of occurance
E.coli	34	15	49 (65.3%)
K. oxytoca	12	3	15 (20.0%)
K. pneumoniae	4	7	11 (14.6%)
Enterobacter spp	10	3	13 (17.3%)
C. diversus	10	4	14 (18.6%)
C. freundii	7	6	13 (17.3%)
Shigella spp	2	1	3 (4.0%)
Salmonella Typhi	4	1	5 (6.6%)
Salmonella spp	2	0	2 (2.6%)
P. vulgaris	5	0	5 (6.6%)
P. mirabilis	3	1	4 (5.3%)
S. aureus	7	6	13 (17.3%)

4.3 Antibiotic susceptibility pattern of isolates

Antibiotic susceptibility test were perform for bacterial isolates by using Kirby-Bauer disk diffusion method. The antibiotics used were Tetracycline (T), Co-trimoxazole (CO), Amikacin (Ak), Ofloxacin (OF), Chloramphenicol (C), and Nalidixic acid (NA)

4.3.1 Antibiotic susceptibility pattern of *E. coli*

E. coli isolates were found to be highly susceptible to Ofloxacin followed by Amikacin, Cotrimoxazole, Chloramphenicol and Nalidixic acid.

Table 6: Antibiotic susceptibility pattern of *E. coli*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	37	75.5	8	16.3	4	8.1
Ofloxacin	49	100	0	0.0	0	0.0
Chloramphenicol	36	73.4	5	10.2	8	16.3
Nalidixic Acid	35	71.4	4	8.2	10	20.4
Tetracycline	29	59.2	7	14.3	13	26.5
Amikacin	44	89.7	5	10.2	0	8.1

n= no of isolates

4.3.2 Antibiotic susceptibility pattern of *Klebsiella oxytoca*

Klebsiella oxytoca isolates were found to be highly susceptible to Ofloxacin, followed by Cotrimoxazole, Nalidixic acid, Chloramphenicol and Amikacin.

Table 7: Antibiotic susceptibility pattern of K*lebsiella oxytoca*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	12	80.0	2	13.3	1	6.7
Ofloxacin	15	100	0	0.0	0	0.0
Chloramphenicol	11	73.3	3	20.0	1	6.7
Nalidixic Acid	12	80.0	0	0.0	3	20.0
Tetracycline	9	60.0	2	13.3	4	26.7
Amikacin	11	73.3	3	20.0	1	6.7

n= no of isolates

4.3.3 Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Klebsiella pneumonia isolates were found to be susceptible to Ofloxacin, Chloramphenicol, Cotrimoxazole, Nalidixic acid and Amikacin.

Table 8: Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	10	90.9	0	0.0	1	9.1
Ofloxacin	11	100	0	0.0	0	0.0
Chloramphenicol	10	90.9	1	9.1	0	0.0
Nalidixic Acid	9	81.8	2	18.1	0	0.0
Tetracycline	6	54.5	0	0.0	5	45.5
Amikacin	8	72.7	3	27.3	0	0.0

n= no of isolates

4.3.4 Antibiotic susceptibility pattern of *Enterobacter* spp.

Enterobacter spp isolates were found to be susceptible Ofloxacin, Cotrimoxazole, Chloramphenicol, Nalidixic Acid and Amikacin.

Table 9: Antibiotic susceptibility pattern of *Enterobacter* spp.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	11	84.6	0	0.0	2	15.4
Ofloxacin	13	100	0	0.0	0	0.0
Chloramphenicol	10	76.9	2	15.4	1	7.7
Nalidixic Acid	8	61.5	3	23.1	2	15.4
Tetracycline	7	53.8	3	23.1	3	23.1
Amikacin	8	61.5	4	30.8	1	7.6

n= no of isolates

4.3.5 Antibiotic susceptibility pattern of *Citrobacter diversus*

Citrobacter diversus isolates were susceptible to Cotrimoxazole, Ofloxacin, Amikacin Chloramphenicol and Tetracycline.

Table 10: Antibiotic susceptibility pattern of *Citrobacter diversus*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	14	100.0	0	0.0	0	0.0
Ofloxacin	14	100.0	0	0.0	0	0.0
Chloramphenicol	10	71.4	2	14.3	2	14.3
Nalidixic Acid	7	50.0	3	21.4	4	28.6
Tetracycline	10	71.4	1	7.1	3	21.4
Amikacin	12	85.7	1	7.1	1	7.1

n= no of isolates

4.3.6 Antibiotic susceptibility pattern of *Citrobacter freundii*

Citrobacter freundii isolates were susceptible to Ofloxacin, Chloramphenicol, Amikacin and Cotrimoxazole.

Table 11: Antibiotic susceptibility pattern of *Citrobacter freundii*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	10	76.9	1	7.7	2	15.4
Ofloxacin	13	100.0	0	0.0	0	0.0
Chloramphenicol	11	84.6	2	15.4	0	0.0
Nalidixic Acid	9	69.2	3	23.1	1	7.7
Tetracycline	9	69.2	3	23.1	1	7.7
Amikacin	10	76.9	1	7.7	2	15.4

n= no of isolates

4.3.7 Antibiotic susceptibility pattern of *Shigella* spp.

Shigella spp isolates were found to be highly susceptible to Ofloxacin, Chloramphenicol followed by cotrimoxazole, nalidixic acid, tetracycline.

Table 12: Antibiotic susceptibility pattern of *Shigella* spp.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	2	66.7	0	0.0	1	33.3
Ofloxacin	3	100.0	0	0.0	0	0.0
Chloramphenicol	3	100.0	0	0.0	0	0.0
Nalidixic Acid	2	66.7	0	0.0	1	33.3
Tetracycline	2	66.7	1	33.3	0	0.0
Amikacin	1	33.3	2	66.7	0	0.0

n= no of isolates

4.3.8 Antibiotic susceptibility pattern of *Salmonella spp*

Salmonella spp isolates were susceptible to Chloramphenicol, Ofloxacin, Cotrimoxazole, Amikacin.

Table 13: Antibiotic susceptibility pattern of *Salmonella spp*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	2	100.0	0	0.0	0	0.0
Ofloxacin	2	100.0	0	0.0	0	0.0
Chloramphenicol	2	100.0	0	0.0	0	0.0
Nalidixic Acid	1	50.0	1	50.0	0	00.0
Tetracycline	1	50.0	0	00.0	1	50.0
Amikacin	2	100.0	0	0.0	0	0.0

n= no of isolates

4.3.9 Antibiotic susceptibility pattern of *Salmonella* Typhi

Salmonella Typhi isolates were susceptible to Cotrimoxazole, Amikacin, Chloramphenicol and Nalidixic acid.

Table 14: Antibiotic susceptibility pattern of *Salmonella typhi*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	5	100	0	0.0	0	0.0
Ofloxacin	3	60.0	2	40.0	0	0.0
Chloramphenicol	4	80.0	1	20.0	0	0.0
Nalidixic Acid	4	80.0	0	0.0	1	20.0
Tetracycline	2	40.0	1	20.0	2	40.0
Amikacin	5	100	0	0.0	0	0.0

n= no of isolates

4.3.10 Antibiotic susceptibility pattern of *Proteus vulgaris*

Proteus vulgaris was highly susceptible to Ofloxacin, followed by Chloramphenicol, Amikacin. Cotrimoxazole, Nalidixic acid and Tetracycline.

Table 15: Antibiotic susceptibility pattern of *Proteus vulgaris*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	3	60.0	2	40.0	0	0.0
Ofloxacin	5	100.0	0	0.0	0	0.0
Chloramphenicol	4	80.0	1	20.0	0	0.0
Nalidixic Acid	3	60.0	2	40.0	0	0.0
Tetracycline	3	60.0	1	20.0	1	20.0
Amikacin	4	80.0	1	20.0	0	0.0

n= no of isolates

4.3.11 Antibiotic susceptibility pattern of *Proteus mirabilis*

Proteus mirabilis (4 isolates) was susceptible to Ofloxacin followed by Amikacin Cotrimoxazole, Nalidixic acid.

Table 16: Antibiotic susceptibility pattern of *Proteus mirabilis*

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Cotrimoxazole	3	75.0	1	25.0	0	0.0
Ofloxacin	4	100.0	0	0.0	0	0.0
Chloramphenicol	2	50.0	1	25.0	1	25.0
Nalidixic Acid	3	75.0	0	0.0	1	25.0
Tetracycline	2	50.0	0	0.0	2	50.0
Amikacin	3	75.0	0	0.0	1	25.0

n= no of isolates

4.3.12 Antibiotic susceptibility pattern of *Staphylococcus aureus*

Staphylococcus aureus (13 isolates) were susceptible to Methicillin and Penicillin least to Cotrimoxazole.

Table 17: Antibiotic susceptibility pattern of Staphylococcus aureus

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Penicillin	12	92.3	0	0.0	1	7.7
Erythromycin	9	69.2	1	7.7	3	23.1
Cotrimoxazole	7	53.8	2	15.4	4	30.8
Methicillin	13	100.0	0	0.0	0	0.0

n=no of isolates

Table 20: Antibiotic sensitivity pattern of gram negative bacterial isolates.

S.N	Antibiotics	Sensitive		Intermediate		Resistance	
		Isolates	%	Isolates	%	Isolates	%
1	Cotrimoxazole	109	81.3	14	10.4	11	8.2
2	Ofloxacin	132	98.6	2	1.4	0	0.0
3	Chloramphenicol	103	76.8	18	13.4	13	9.7
4	Nalidixic Acid	94	70.1	17	12.6	23	17.1
5	Tetracycline	81	60.4	19	14.2	34	25.4
6	Amikacin	108	80.6	20	14.9	6	4.4

Table 21: Antibiotic susceptibility pattern of gram positive isolates:-

Antibiotics	Sensitive		Intermediate		Resistant	
	isolates	%	isolates	%	isolates	%
Penicillin	12	92.3	0	0.0	1	7.7
Erythromycin	9	69.2	1	7.7	3	23.1
Cotrimoxazole	7	53.8	2	15.4	4	30.8
Methicillin	13	100.0	0	0.0	0	0.0

CHAPTER- V
DISCUSSION

In the study, a total of 45 raw buff meat samples, 5 from each 9 different locations Byasi, Kamalbinayak, Chayamasingh, Sukuldhoka, Suryamadhi, Bholachee, Barahisthan, Thimi, and Lokanthali were randomly analysed. The mean total plate count ranged from $1.9x10^6$cfu/gm to $1.4x10^7$cfu/gm. The lowest count was found to be from Barahisthan and the highest count was found to be from Kamalbinayak.

In this study, it was found that the raw buff meat of Lokanthali showed the mean total plate counts of $6.2x10^6$ cfu/gm, sample from Thimi $4.5x10^6$cfu/gm, Sukuldhoka $1.1x10^7$ cfu/gm, Suryamadhi $5.7x10^6$ cfu/gm, Barahisthan $1.9x10^6$ cfu/gm, Chayamasingh $1.0x10^7$ cfu/gm, Kamabinayak $1.4x10^7$ cfu/gm, Byasi $6.4x10^6$cfu/gm and Bholachee $3.6x10^6$ cfu/gm..

The higher incidence of microbial load in buff meat obtained in this study might be attributed to unhygienic and improper handling of animals during slaughter, dressing and evisceration. The usual practice of washing the carcass with the same water in which intestines and offal had been washed might be one of the predominant reasons for increased microbial counts of the meat. A complete ignorance on the part of the meat handlers/ butchers in hygienic handling of carcasses during slaughter and retailing processes might be the main factors for producing meat with high microbial loads (Mukhopadhyay *et al.,* 2009). Refrigerators are not commonly available in every household in developing countries. In addition, frequent interrupted power supply is a day to day problem in countries like Nepal. Under these situations, people held the buff meat at ambient temperature hence the microbial load of meat may increase (Kandeepan *et al.,* 2010**).**

In the study, a total of 30 buff sausages samples collected from minimarket, butchner and fast food shop were randomly analysed. The mean total plates count ranged from $1.5x10^5$cfu/gm to $8.3x10^5$cfu/gm. The lowest count was found to be from sausages sample collected from minimarket and the highest count was found to be from sausages sample collected from butchner.

It was found that the buff sausages sample collected from minimarket show the mean total plate counts of $1.5x10^5$cfu/gm, buff sausages sample collected from butchner $8.3x10^4$cfu/gm and buff sausages sample collected from fast food shop ranged $7.5x10^5$cfu/gm.

The high bacterial counts from the buff sausages collected from butchner in this study is generally attributed to the filthy environment, poor personal hygiene of the processors, retailers, inadequate storage and thawing conditions, contamination from grinder and the time between mincing and mixing and the use of contaminated utensils during processing, packaging (Koffi. Nevry *et al*., 2011; Gongor and Gokoglu, 2010). There could be possible cross contamination of the finished product from adjacent raw meat through unclean hands of the handlers and/or flies.

Coliform are used as an indicator of post processing contamination of meat. High count indicates growth has occurred. Enterobacteriacea forms part of the coliform group and are primary environmental saprophytes often found in the intestinal tract of man and lower animals. They are very important pathogens and because of their association with the intestinal tract they easily become introduced into food either during slaughter of animals or subsequent processing and food handling. These enteric pathogens may grow in foods and cause food infections after ingestion by attacking the intestinal walls, causing symptoms of nausea, vomiting, pain, diarrhoea and headache.

In the study, it was found that the buff meat of Lokanthali show the mean total coliform counts of $1.0x10^5$cfu/gm, sample from Thimi showed $6.7x10^4$cfu/gm, Sukuldhoka $1.1x10^5$cfu/gm, Suryamadhi $6.3x10^4$cfu/gm, Barahisthan $6.5x10^4$cfu/gm, Chayamasingh $1.3x10^6$cfu/gm, Kamalbinayak $1.2x10^6$cfu/gm, Byasi $1.0x10^6$cfu/gm and Bholachee $5.5x10^5$ cfu/gm

The high coliform count from raw buff meat collected from Chayamasingh may be due to poor personnel hygiene of the butchner and retailers such as careless sneezing and coughing, handling of the meat and the money with the same unwashed hands, cross contamination between adjacent meat through unclean hands of the handlers and /or flies, reuse of washing water having high load of organism during handling, washing, filthy environment and the contamination of the organism from the animal gut during processing, cutting and selling (Koffi-Nevry *et al*., 2011; Salihu *et al*., 2010)

It was found that the buff sausages sample collected from minimarket show the mean total coli form counts of $1.3x10^3$ cfu/gm, buff sausages sample collected from butchner $1.8x10^4$ cfu/gm and buff sausages sample collected from fast food shop $3.5x10^4$ cfu/gm.

It was found that raw buff sausages sample collected from fast food shop found to be having the highest coliform count. The high number of coliform count might be due poor personal hygiene of retailer, poor quality of sausages, reuse of water for washing plates inadequate storages and thawing condition. Poor personal hygiene practices such as negligence to wash hands after visiting the bathroom may result in up to 10^7 pathogens under the finger nails of the food handlers.

The total aerobic count and total coliform count were higher in the raw buff meat than in the raw buff sausages samples. These differences may be explained by the microbial quality of ingredients used and personal hygiene.

Enterobacteriacea forms part of the coliform group and are primary environmental saprophytes often found in the intestinal tract of man and lower animals. They are very important pathogens and because of their association with the intestinal tract they easily become introduced into food either during slaughter of animals or subsequent processing and food handling. These enteric pathogens may grow in foods and cause food infections after ingestion by attacking the intestinal walls, causing symptoms of nausea, vomiting, pain, diarrhoea and headache.

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. Traditionally these genera included *Escherichia, Citrobacter, Enterobacter* and *Klebsiella*. However, based on the modern taxonomical criteria, the group is heterogeneous and includes non-fecal lactose fermenting bacteria as well as other species which are rarely found in feces but are capable of multiplication in water.

The bacterial isolates isolated from this study were identified as *E. coli, K. oxytoca, K. pneumoniae, Enterobacter* spp, *C. diversus, C. freundii, Shigella* spp, *Salmonella* Typhi, *Salmonella* spp, *P. vulgaris, P. mirabilis* and *S. aureus* by comparing their morphological and biochemical characteristic. Their frequency of occurrence in the examined meat samples is presented in Table 5. The presence of these organisms could be attributed to the fact that meat contains an abundance of nutrient required for the growth of microorganism. (Magnus, 1981)

Altogether 8 different genera of bacteria were isolated from raw buff meat samples of 9 different locations of Bhaktapur. Among 100 bacterial isolates, *E. coli* (34.0%) was the predominant organism followed by *K. oxytoca* (12.0%), *C. diversus* and *Enterobacter* spp (10.0%), *C. freundii* and *S. aureus* (7.0%), *P. vulgaris* (5.0%), *Salmonella* Typhi and *K. pneumoniae* (4.0%) and *P. mirabilis*, *Salmonella* spp and *Shigella* spp (2.0%).

Altogether 8 different genera of bacteria were isolated from 19 samples of buff sausages. Among the 47 isolated bacteria, *E. coli* (37.9%) was predominant

organism followed by *K. pneumonia (14.8) Citrobacter freundii* and *Staphylococcus aureus* (12.7%), *Citrobacter diversus* (8.5%), *Enterobacter* spp and *K. oxytoca* (6.3%) *Salmonella* Typhi, *Shigella* spp and *P. mirabilis* (2.1%).

The presence of *E. coli* depicts a state of poor hygienic and sanitary practices employed in the slaughtering, processing and packing of fresh meats. Most of the organisms found in this study are those commonly found in the soil and water. The presence of *E. coli* and *Enterobacter* spp is an indicator of faecal contamination of meat. This might be due to possible contamination of fresh meat and meat product itself during slaughtering or processing or unhygienic handling of the meat right from slaughtering or due to contamination from the skin, mouth, nose of the handlers which can be introduced directly to the foods by meat handlers with lesions caused by *S. aureus* on hand and arms coming into contact with foods or by coughing and sneezing (Okonko *et al*., 2008). The isolation of *Enterobacter* spp may be a result of poor environmental condition due to dust and contamination of the water used during slaughtering (Omoruyi *et al.,* 2011). Though organisms identified like *E. coli* is normal inhabitant of the intestine and most strains are non pathogenic but its presence indicates possibility of harmful pathogens causing several foodborne diseases showing faecal contamination.

The presence of respiratory pathogen such as *Klebsiella pneumoniae* in meat and meat products might be attributed to the bacterial aerosols generated due to sneezing and coughing in public places. Presences of *Klebsiella* spp may be due to poor personal hygiene of the meat handler, unhygienic handling of food etc (Biswas *et al.*, 2011)

Salmonella spp. is the causative agents for typhoid, the lethal disease that cause mortality and morbidity in a large fraction of the people of Nepal and other under developed South Asian countries. Its presence is very alarming as it can cause food borne infections to the consumers. *S.* Typhi is normally found only in humans, although *Salmonella* spp is found in domestic animal in rare occasion

(Joshi DD, 2005). Salmonellosis is due primarily to foodborne transmission because the bacteria infect beef and poultry and are capable of growth on the food. Since the route of transmission is fecal-oral, any food contaminated with feces may transmit the organism to new host.

Presence of *Shigella* indicates contamination of meat and meat products with water polluted with human sewages. *Shigella* causes shigellosis in human. Most cases of shigellosis are the result of person to person transmission through the faeco-oral route. Detection of *Shigella* spp indicates waterborne contamination of meat and meat product (Cheesebrough M, 1984).

Staphylococcus aureus are normal flora in human and animals, their presence in foods are indications of excessive human handling (Adamolekun and Adamolekun, 1992). It is a common environmental contaminant and could be introduced into meat and meat product through cross contamination, utensils used for serving food or food preparation surfaces and even from handler hands. They are habituated in warm, damp and congenital atmosphere of the nose, throat, in the pores and hair follicle of the skin and on the surfaces of skin. *S. aureus* can be transferred to meat during processing, packaging, preparation and service by touching, breathing, coughing or sneezing. *S. aureus* can also establish itself on food processing equipment and so food can become contaminated during processing (Shapton and Shapton, 1991). Its presence in the samples might be due to contamination from the working environment of the meat preparation and the sale point.

In a few similar studies, Prasai P (2000) reported the presence of *E. coli, Bacillus spp, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Enterobacter aerogens, Salmonella spp, Klebsiella oxytoca, Streptococcus faecalis, Citrobacter diversus, Providencia rettgeri, Citrobacter freundii, Proteus mirabilis,* and *Enterobacter cloacae* in 14 raw buff meat sample of Kathmandu Valley.

Nafisa *et al* (2010) showed the raw meat in retail shops in Karachi Pakisthan were contaminated with high load of pathogen viz *E.coli* O157.H7, *Listeria* spp, *Salmonella, Enterobacter, Shigella* spp, *Staphylococcus aureus Brucella,* and *Klebsiella spp* which corroborated with our findings.

Phillips *et al*. (2001) reported the presence of *Escherichia coli* in 5.1%, *Salmonella* in 0.1% and of coagulase positive staphylococci in 17.5% of 990 cartons of boneless beef.

Phillips *et al*. (2006) examined 1,155 beef carcass and 1082 frozen boneless beef, *Salmonella* was detected in 1 sample of frozen boneless beef whereas *E. coli* O157:H7 was isolated from 1 of 1,143 carcasses. Coagulase positive staphylococci were isolated from 28.7% of beef carcasses and 20.3% of boneless beef samples

Joshi *et al.* (2005) reveal the presence of coliforms especially *E. coli* in 89% of buff meat sample (number of sample process=37) and detection of *Salmonella* Typhi, *S. choleraesius* and *Salmonella* of subgeneus I and II group in 5 sample of buff meat. The detection of *Salmonella* spp may be the cases of extrinsic contamination.

Other studies Ismul Nazmul *et al.* (2006) reported the detection of *E. coli, Streptococcus faecalis, Staphylococcus aureus,* and *Salmonella* in buffalo meat sold at retail shop in Alighar and Iroha (2011) reported the detection of *Bacillus cereus, E. coli, Klebsiella spp, Pseudomoas aeruginosa, Shigella dysenteries,* and *Staphylococcus aureus* in raw beef meat sold in Abakaliki Ebonyi State Nigeria.

Althogether 6 different antibiotic discs namely Co-trimoxazole (25 µg), Ofloxacin(5µg) Chloramphenicol (30µg), Amikacin (30 µg), Tetracycline (30 µg), Nalidixic acid (30µg) were used for the antibiotic susceptibility test of gram negative isolates and 5 different antibiotic disc namely Methicillin, Penicillin,

Erythromycin, and Co-trimoxazole for gram positive (*S. aureus*). 134 gram negative isolates and 13 gram positive isolates were analysed. Among the gram negative isolates resistance was found most commonly directed toward. Tetacycline (25.4%) followed by, Nalidixic acid (17.1%), Chloramphenicol (9.7%), Cotrimoxazole (8.2%), Amikacin (4.4%). The antibiotics disc used for the antibiotics sensitivity test of gram negatives isolates; Ofloxacin was most effectives with 98.6% efficacy followed by Cotrimoxazole 81.3%, Amikacin 80.6%, Chloramphenicol 76.8%, Nalidixic acid 70.1%, and Tetracycline 60.4%. Among *S. aureus* resistance was found most commonly directed toward Cotrimoxazole (30.8%), Erythromycin (23.1%), and Penicillin (7.7%). No Methicillin resistances *S. aureus* were observed. Thus for gram positive isolates Methicillin was most effective with 100% efficacy followed by Penicillin 92.3%, Erythromycin 69.2% and Cotrimoxazole 53.8%.

Most of the antibiotics used in antibiotics susceptibility test were found to be effectives for the tested bacterial isolates. Only few isolates showed resistance to the tested antibiotics disc. The main reason for the emergence of antibiotics resistance may be due to indiscriminate use of antibiotics in animal's feed and treatment. The use of antibiotics has been proven to be effective means for the prevention and control of bacteria infection, but their indiscriminate use can have adverse consequences by promoting the selection and prevalence of drug resistant microbial populations (Braude, 1978). The problem may be due to the natural resistance of species to certain antibiotics (Allison and Gilbert, 1995), possible transfer of antibiotic resistance among species, and the use of sub-therapautic doses of antibiotics in animal feeds to improve animal productivity, which could also select for resistant strains. Paddock (1996) suggested 3 possible ways in which the use of antibiotics could pose a risk to human health and these include: i) antibiotics resistant pathogens in animal are selected, food products then become contaminated during slaughter and or food preparation, the food is then ingested causing infection which requires antibiotic therapy and therapy is then compromised due to resistant strains; ii) resistant non pathogenic bacteria are

selected in animals transferred to humans via consumption of contaminated food products and resistant genes are subsequently transferred to other bacteria in the gut; iii) antibiotics which may remain as residues in animal products such as meat and milk can also lead to the selection of resistant bacteria in the consumer of the food products.

The meats sold in markets are grossly contaminated with coliform bacteria as well as other possible pathogenic gram negative organism and gram positives organism. The possible source of contaminants are due to the unhygienic manner of handling meat, the environment upon which the animal is slaughtered as well as water used in the processing of the meat. This also implies that these meats are viable source of various diseases.

CHAPTER-VI
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The total plate count and total coliform count of raw buff is found higher than that of the raw buff sausages. Out of 45 raw buff meat sample 41(91.1%) raw meat sample and of 30 buff sausages, 19 (63.3%) buff sausages were found to be contaminated with coliform. The bacteria isolated from the raw buff meat and raw buff sausages are the potent human pathogen which are responsible for foodborne illness. From the study it can be concluded that the microbial quality of the raw buff meat is poor and unacceptable for the human consumption due to high microbial load. This indicates potential risk of infection for the consumer by different types of food borne disease. Unscientific slaughtering, poor personal hygiene, poor sanitation, use of polluted water during washing of carcasses, and the contamination of the meat from the intestinal contain during processing, cutting and selling were major source of contamination.

The study showed that *E. coli* was the most predominant organism in the both raw buff sausages and raw buff meat which is the faecal indicator organisms and presence of it's indicates that the quality of meat and sausages is relatively poor. Beside *E. coli*, *S. aureus*, *Salmonella* spp, *Shigella* spp (causative agent of food borne illness) were isolated which indicated meat and sausages are handle unhygienic. The other organism are mainly of Enterobacteriacae family and pathogenic bacteria to human being and bacterial load are relatively high in the raw buff meat indicating the quality of meat in Bhaktapur is microbiologically poor.

Since contaminated raw meat is one of the main sources of food borne illness, it may require to prevent the contamination of the meat by applying HACCP and good manufacturing practices, appropriate hygienic measures including proper washing of carcasses, storage and finally processing of meat

6.2 Recommendations:-

Following recommendations are given:-

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a. Since the meat and sausages contain high microbial load, there should be the use of refrigerator in order to check the microbial growth and use of the net for the protection from the flies.
b. Most of the microorganism isolated from the meat and sausages are the commensal of the intestinal tract of buffaloes so selling of meat along with animal gut or intestinal part should be avoided.
c. Raw meat and raw sausages should be well cooked before consumption
d. Good personnel hygiene should be maintained while handling the meat and meat products.

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