CHAPTER –I
INTRODUCTION AND OBJECTIVES

1.1 Background

Nepal is the agricultural country. Agriculture contributes 38% of the nation's GDP and livestoke contributing almost 11% to GDP (World Bank, 2002). Livestoke population in Nepal for 2004/2005 was 4.08M buffaloes. Roughly 70% of households keep some type of livestock's including cows, buffaloes, pigs and chicken (FAO, 2005). Buffalo is one of the major livestock species for milk and meat production, constitutes two thirds of meat consumption in Nepal (TLDP, 2002).

Meat is the most perishable food consumed by most of the people of this universe from very beginning of human civilization till now. The word meat comes from the old English word mete which referred to food in general. Meat, in its broadest definition, is animal tissue used as food. Meat is widely used to define flesh and offal including their natural associate's skin and gristle, derived from carcass of any animal and bird normally used for human consumption. Because of essential nutrients it contains meat eaten in moderate amounts can be considered a valuable component for flavor and taste since meat helps to make eating a joyful experience.

There is a shortage of meat especially in the cities of Nepal, which is filled by the importation of animals from neighboring countries (TLDP, 2002). About 0.3 millions buffaloes are annually imported from India alone (DLS, 2002). Kathmandu has the country's largest per capital consumption of meat. Each resident consumes 18kg of a meat a year, whereas the average Nepali eats only 10 kg way below the worldwide average of 40kg per person per year.

Meat consumption varies widely while comparing different countries as it depends on factors like socioeconomic condition, religious beliefs, cultural practices, etc. In a resource poor and developing country like Nepal, natives have included meat as important part of their diet to supplement nutritional requirements. In Nepal buffaloes, contribute about 64% of buffaloes meat consumed, followed by goat meat 20%, pork 7%, chicken 6% and sheep 3% (Joshi et al., 2001).

The nutrients consist of protein, amino acids, vitamins B6, B12, folic acid, niacin, essential fatty acids and minerals like Zinc, Iron, etc. Vitamin B12 is found in the meat but not in plants and is the important source of pernicious anemia. Foods from the plant origin provide moderate level of vitamins, protein and minerals. On the contrary, meat is a good source of protein vitamin B6, B12, folic acid, niacin, essential fatty acid and minerals like zinc iron etc. Meat from different animals provide different grade of nutrients.

Due to its nutrients content, meat is an excellent media for the growth of pathogenic organisms. Meat and other meat products contribute organisms that contribute significantly to high incidence of food born diseases. In Nepal, sub-tropical climate, poor sanitary condition, improper handling, poor food hygienic practices and lack of prevention against diseases had used a number of diseased to erupt from the meat source. Bryan (1973) listed approximately 200 meat diseases that can be transmitted to man by food. The list of pathogens which can be transmitted from animals to human by food contained about 16 kinds of bacteria, 3 groups of viruses, 22 parasites and 5 protozoa (Singh and Koulikovski, 1995).

Commercial ground meat generally consists of trimming from various cuts and thus represents pieces that have been handled excessively generally contain high levels of microbial contaminations. Ground meat provided 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. In some commercial establishments, the meat grinders, cutting knives, and storage utensils are rarely cleaned as often and as thoroughly as is necessary to prevent the successive build up of microbial numbers. One heavily contaminated piece of meat is sufficient to contaminate others, as well as the entire lot, as they pass though the grinder (James, 1970).

It is generally agreed that the internal tissues of healthy slaughter animals are free of bacteria at the time of slaughtering, assuming that the animals are not in a state of exhaustion. When one examines fresh meat and poultry at the retail level, different numbers and types of microorganisms are found in fresh meat which is due to the stick knife, animal hide gastrointestinal tract, hands of handlers, containers, handling

and storage environment, lymph nodes, etc. The most significant of the above are non-sterile containers (James et al., 2005).

The most common bacteria occurring on fresh meat includes: *Pseudomonas* species, *Staphylococcus* species, *Micrococcus* species, *Enterococci* species and Coliforms. The low temperature at which fresh meat is held favors the growth of psychrophilic microorganisms. The surface of raw meat is contaminated with variety of microorganisms. It is desirable to maintain a very low microbial level of contamination on raw meat. The presence of extremely large numbers of microorganisms suggests that meat have been contaminated and that the meat and meat products are indeed susceptible to further deterioration.

A slaughter house, also called an abattoir is a place where animals are killed and processed into meat products. The design, process and location of slaughter house respond to a variety of concerns. Slaughtering animals on a large scale process has significant logistical problems and public health concerns.

In Nepal, lack of appropriate slaughtering facilities and unsatisfactory slaughtering technique are causing unnecessary losses in meat as well as valuable by products. Animal's particularly buffaloes are slaughtered in Kathmandu in slaughtering places which are frequently polluted with street dust, garbage, human excreta, animal blood, intestinal contents and dirty effluents and which are not protected against dogs, rodents and insects. Meat products under such conditions are generally deteriorated due to bacterial infections and which cause food poisoning time to time. Due to lack of meat inspection, meat from the unhealthy and parasitic infected animals is a source of infection to human as well as to animals. Besides, meat quality is adversely affected by careless handling condition in the slaughtering places as well as in the meat markets or shops (Joshi, 1991).

Surface slime on meat is usually caused by *Pseudomonas spp* and *Acinetobacter spp,* but *Streptococcus, Lactobacillus, Leuconostoc* and *Micrococcus* may also be responsible.

Pseudomonas and *Clostridium* may cause Putrefaction. The lactic acid bacteria on the other hand can cause souring due to the production of organic acids (Harrigan and Lance, 1976).

Coliforms are gram-negative asporogenous rods that ferment lactose within 48 hours and produce dark colonies with a metallic sheen on Endo-type agar. Coliforms are represented by four or five genera of the family *Enterobactericeae* and *Klebsiella, Citrobacter*. Species have been referred to as intermediate coliforms, and delayed lactose fermentation by some strains.

Coliforms grow well on a large number of media and in many foods. They grow at temperatures as low as -2^0C and as high as 50^0C. In foods, growth is poor or very slow at 5^0C, although the growth of coliforms has been reported at 3-6^0C. Coliforms have been reported to grow over a p^H range of 4.4-9.0. *E.Coli* can be grown in a minimal medium containing only an organic carbon source such as glucose and produce visible colonies within 12-16 hours at 37^0C. They can be expected to grow in a large number of foods under the proper conditions.

Coliforms can grow in the presence of bile salts, which inhibit the growth of gram-positive bacteria. Unlike most other bacteria, they have the capacity to ferment lactose with the production of gas. Buttiaux and Mosses concluded that various pathogens may persist after *E.coli* is destroyed in foods that are frozen, refrigerated, or irradiated.

Salmonella are gram negative rods. They are all motile except *Salmonella gallinarum* and *Salmonella pullorum*. Most *salmonellae* are found in the intestine of animals. *S.* Typhi and *S.*paratyphi are excreted in the faeces and urine of infected patients and are present in the gall bladder of long term carriers. Infection may occur by ingesting the organisms in contaminated food or water, or from contaminated hands. *S.*Typhi is spreads mainly by water and *S.*paratyphi A and B by food (Cheesbrough, 1984).

The primary habitat of *E.coli* is the intestinal tract of most warm-blooded animals, although sometimes it is absent from the gut of hogs. The primary habitat of *Enterobacter aerogens* is vegetation, and occasionally, the intestinal tract. It is not

difficult to demonstrate the presence of coliforms in air and dust, on the hands and in and on many foods. Although the presence of large numbers of coliforms and *E.coli* in foods is highly undesirable, it would be virtually impossible to eliminate all from fresh and frozen foods. Low numbers of coliforms are permitted in sensitive foods at numbers ranging from 1 to not over 100/g or 100 ml. These criteria reflect both feasibility and safety parameters. For poultry products, coliforms are not good sanitary indicators because salmonellae may exist in a flock prior to slaughter and thus positive faecal coliforms tests may be unrelated to post slaughter contamination.

Meat is one of the daily needs for human life and is necessary to find out the microbial quality of meat and meat products before consumption. Raw meat may be contaminated from various sources which may consist various microorganisms that are detrimental to human health. This study is targeted to find out the microbial quality of raw meat. Furthermore it is absolute pattern of the isolates because these bacteria can contaminate the meat product from various cases i.e. it may be natural microbial pool or from different hospital or industrial environment in which the organism may show antibiotic resistance. Thus this study also targets to explore antibiotic succeptibility patterns of the isolates which would be helpful to reduce the chances of contamination caused and eventual problems created by them.

1.2 OBJECTIVES

To describe the occurrence and distribution of bacteria in buff meat and their succeptibility to common antibiotics.

Specific Objectives

1. To determine the total mesopholic bacterial count of buff meat.
2. To enumerate the total coliform count of buff meat.
3. To detect gram negative potential pathogens with special reference on *Salmonella* species.
4. To describe antibiotic susceptibility pattern of isolated gram negative bacteria.

CHAPTER–II
LITERATURE REVIEW

The trend towards functional foods has led to the publication of several articles describing studies of the effects of including 1 or more ingredients with functional properties in various types of food, within which meat and meat products deserve special attention. The object of including functional ingredients in the case of meat is not only concerned with providing it with certain desirable properties but also an attempt to change its image in these health conscious days.

The underlying idea behind functional food is to reduce the prevalence of chronic disease by curbing the composition of habitually consumed foods. The formulation of foods according to the beneficial effects that their non-nutritional ingredients may have for the consumer has become as area of great interest for large food companies, including meat sector (Vasconocellas, 2001)

2.1 Quality of Buff Meat

Meat is an important source that contains trace of several different vitamins, proteins, minerals and essential fatty acids etc, but meat is also a primary source of many bacteria responsible for food borne infection and intoxications. Organisms found in the live animals can be carried through raw meats after slaughtering that may persists through further processing and ultimately may appear in the final retailer product if insufficient attention is paid.

Table 1: Grade of nutrients provided from different animals meat:

	BEEF	BUFF	GOAT	MUTTON	PORK
PROTEIN (mg/100g)	22.6	19.4	21.4	18.5	18.7
FAT(g/100g)	2.6	0.9	3.6	13.3	4.4
MINERAL (mg/100g)	1.2	1.0	1.1	1.3	1.0
MOISTURE (%)	74.3	78.7	74.2	71.5	77.4

Frazier and Westhoff (1978)

Joshi et al. (2003) found that the meat is an important source of protein and valuable commodity in resource-poor communities. In many develop countries, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses of meat as well as in valuable by products from animal carcasses. Animal slaughtering and meat inspection Act mandates slaughter house construction and meat inspection and control. For improvement in animal slaughtering and meat inspection in both rural and urban areas of Nepal, several strategies are recommended.

Greenberg et al. (1966) studied the incidence of *Mesophilic Clostridium* spores in raw meats in processing plants in United States/Canada and found a mean P.A. spore count/gram of 2.8 from 2385 meat samples. Of the 19,727 P.A. spores isolated, only one were a *Clostridium botulinum* spore, and it was recovered from chicken. The large number of meat samples studied by the investigators consisted of beef, pork, and chicken obtained form all parts of the United States and Canada. The significance of the P.A. spores in meat were due to the problems encountered in the heat destruction of these forms in the canning industry.

Abdul and Chempaka (1995) concluded from a survey on the nutritional value of some processed meat products in Malaysia more than double from 15.70 kg in 1970 to 35.71 kg in 1990. This increase in meat consumption was mainly due to the rapid development and wide acceptance of value added meat and poultry products among Malaysia consumers. Meat products such as burgers, sausages, and hotdogs are widely accepted and consumed by all ethnic groups at home as well as in the fast food restaurants. In vivo and vitro protein digestibility studies indicated high value on the digestibility of locally manufacture meat products. It was also observed that many by products from the animal industry forms non- conventional sources were increasingly being utilized in the manufacture of processed meat product.

Kandeepan and Biswas (2000) studied on future remedy for the nutritional security of Buffalo meat in India. And found if the quality gets deteriorated, the meat preserved in refrigerator would impact greatly on the health of consumers. Hence meat samples from five year old sixteen buffalo bulls were analyzed in the fresh state (0 day) and after 4 and 7 days in chiller ($4\pm1^{o}C$) and 4, 7, 14, 30, 60 and 75 days in freezer (-

10±1^{o}C) in a domestic refrigerator. The chiller storage increased but freezer decreased the microbial counts (SPC, PC and Coliforms). The values of odour and flavour scores decreased with increasing storage period, whereas, texture, tenderness and juiciness scores showed an increasing trend. Thus it was concluded that a storage period up to 4 days in chiller and 30 days in freezer could satisfactorily maintain the buffalo meat quality.

Sarwar et al. (2009) studied an important contributor to meat in many developing countries. In many parts of Asia, where the buffalo is an integral part of the food chain and rural economy, irregular and inadequate availability of quality feedstuffs and their utilization are hampering the performance of this unique animal. Balanced nutrition and better management can enhance buffalo productivity. Many efforts have been made in the last few decades to improve nutrient supply and utilization in buffaloes. Recent research on locally available feed resources such as crop residues, and industrial by-products, dietary addition of micronutrients, use of performance modifiers and use of ruminally protected fat and protein sources have shown significant potential to improve growth, milk yield and reproductive performance of buffaloes. However, a number of issues, including establishment of nutrient requirements for dairy and beef, development of buffalo calf feeding systems, nutritional management of metabolic and reproductive anomalies, and understanding and exploitation of the buffalo gut ecosystem, need to be addressed. Extensive coordinated research and extension efforts are required for improved buffalo nutrition in developing countries.

Anjaneyulu et al. (2007) studied on meat production and importance of proceeding of value added products are presented. Physico-chemical and functional properties of buffalo meat and their improvement by using polyphosphate and pre blending of meat with salt and food additives were discussed. Processing of committed meat products such as patties, sausages, enrobed cutlets, traditional meat products their packaging were highlighted. Strategies for quality achievement of meat and meat products were proposed in this review.

Kandeepan et al. (2010) reported on the quality of buffalo meat keema at different storage temperature. Here Buffalo Meat from young male spent male and spent

female. The keema prepared by standarized ordered formulation and processing were stored at ambient (37 ± 1^0C) and refrigeration ($a\pm1^0$C) temperature and the quality was compared on day 3. The psychochemical characteristics showed sufficiently higher energy and fat content in keema prepared from spent buffalo groups compared to young males. The buffalo meat keema could be stored 0 up to 2 days at ambient temperature with an overall acceptability in the range of extremely acceptable to moderately acceptable.

Yashoda et al. (2000) Conducted study to assess the microbiological quality of buffalo carcasses processed hygienically in a modern abattoir as against those processed in traditional slaughter units of different sites. The hygienic measures followed include abattoir cleanup operation, processing on overhead rail, careful removal of skin and viscera, closure of esophagus and rectum and washing the carcasses with a spray of clean water. The carcasses, meat cuts and minced meat obtained from both the sources were examined for microbial load. A significant difference in microbial load was seen in carcasses obtained from two sources, with carcass from traditional slaughter units showing 1.0 – 2.50 log higher microbial load. The reduction in microbial load in carcasses processed in a modern abattoir was attributed to the hygienic measures followed. The microbial load was high in shoulder portion and lower in leg portion and the data indicated that samples of shoulder, neck and rib surfaces provide a more realistic estimate of microbial load on buffalo carcasses. The meat cuts obtained from hygienically processed carcass had a shelf-life of six days as against three days for those obtained from traditional slaughter unit. The minced meat from hygienically prepared carcass had a shelf-life of four days as against one day for those obtained from local slaughter unit. The study indicated that by adopting proper hygienic and sanitary practices during processing of buffalo meat would yield meat with acceptable microbiological quality with extended shelf-life.

Nortje et al. (1989) studied on microbiological quality of retail beef meat. They isolated aerobic, psychrotrophic, psendomonadacae, enterobacteriaceae and lactobacillaceae. They found that the good hygienic at the retail level was reflected in

lower counts for all groups of bacteria studied and greater self-life. They also found that aerobic count was generally greater for minced meat.

Bhat and Bhat (2011) reviewed on a scientific program in understanding the relationship between nutrition and health had an increasingly profound impact on consumer's approach to nutrition which had resulted in the development of the concept of functional foods. It is a practical and new approach to achieve optional health status by promoting the state by promoting the state of well being and possible reduce the risk of disease meat and meat products can be modified by adding ingredients considered beneficial for health or by eliminating or reducing components that are considered harmful.

Sachindra et al. (2003) studied on the microbial levels of buffalo sausage during preparation and storage at 4± 1^0C. Microbial counts in raw minced meat were, total plate count (TPC) (log cfu/g) 5.41 ± 0.25; coliforms (MPN/g) 23.2; *Staphylococcus aureus* (log cfu/g) 1.57± 0.11; yeasts and molds (log cfu/g) 2.29 ±0.07 and lactic acid bacteria (LAB) (log cfu/g) 0.60 ± 0.20. Sausage emulsion showed similar trend in microbial counts with minimal microbial contamination during the preparation of emulsion. Cooked buffalo sausage gave the following microbial counts: TPC (log cfu/g) 3.75 ±0.31; coliforms (MPN/g) 0.2; LAB (log cfu/g) 0.07± 0.01; yeast and molds (log cfu/g) 0.72± 0.07. *S. aureus*, *Clostridium perfringens* and *Bacillus cereus* were not detected in cooked sausages. These results indicate that steam cooking for 45 min followed in the study was effective in reducing the microbial counts substantially. The investigation revealed that shelf life of cooked buffalo sausage was 31 days in either vacuum or CO2 at 4± 1^0C. The results indicated that spoilage of vacuum packed cooked buffalo sausage was likely due to LAB while microflora other than LAB may be responsible for spoilage of CO_2 packed cooked buffalo sausage. The study suggests that measures such as low initial microbial counts, hygienic precautions during preparation of sausage, steam cooking for 45 min, vacuum or CO_2 packing and storage at 4 ± 1^0 C would control the microbial growth and provide wholesomeness and safety to the buffalo sausage.

Gurunathan and Kandeepan (2009) studied the comparison of quality and shelf life of buffalo meat Pattie stored at refrigeration temperature. A programmed was

undertaken to compare. The quality of buffalo meat patties made form intensively reared young male semi extensively reared spent male and female groups and stored at refrigeration (4± 1^{o}C).Buffalo meat patties were evaluated for shelf life attributed by analyzing the changes in physicochemical, microbiological, and sensory attributes. The overall acceptability of patties was better in spent buffalo group than young male group. Increasing stage time resulted in significantly higher p^{H}, TBARS, total aerobic mesophiles coliforms, *Staphylococcus* aureus and *Psychrophsilc* counts but decreased appearance, flavour, juiciness, of buffalo meat patties. The patties were well acceptable within 20 days in young male group and 25 days in spent buffalo group respectively during refrigerator storage.

Shekhar and Kumar (2005) studied a total of 512 buffalo samples from slaughterhouses of Mathura, Agra and Aligarh districts of Uttar Pradesh, India, which included meat (musculature), liver, heart, kidney; lymph nodes (mesenteric, iliac and prescapular); swabs from butchers' hands and knives and air samples from surrounding environment of slaughterhouses. These samples were processed for total viable count, coliform count and for the isolation and identification of bacteria. The analysed samples showed the $\log_{10}$ values of mean total viable counts as 6.14, 6.23, 6.12, 6.34 and the mean coliform counts as 4.40, 3.75, 3.25, 3.38 cfu/g for meat, liver, heart and kidney, respectively. 163 isolates of bacteria were identified from these samples, including *Staphylococcus aureus* (29), *Escherichia coli* (23), *Streptococcus pyogenes* (20), *Proteus ammoniae* (17), *Bacillus cereus* (15), *Pseudomonas aeruginosa* (13), *Klebsiella pneumoniae* (11), *Enterococcus faecalis* (9), *Arcanobacterium pyogenes* (8), *Brucella abortus* (6), *Shigella dysenteriae* (5), *Salmonella* spp. (4) and *Corynebacterium renale* (3).

Biswas et al. (2010) focused on food safety in relation to pesticide and veterinary drug residue and myotoxin in meat products. The impacts of these consumers awareness was large concern for the meat industry. These study review the cause of residue in meat, type of residue found, their detection methods,incidence and their regulation with emphasis on public health risk and their assessment.

Stiles and Ng (1981) studied the biochemical characteristics and identification of *Enterobacteriaceae* isolated from meat of 442 meat samples examined, 86% yielded

enteric bacteria with all 127- ground beef samples being positive. The most frequently found were *Escherichia coli*, biotype. I (29%), *Serratia liquefaciens* (17%) and *Enterobacter agglomerans* (12%). A total number of 721 isolates (32%) were represented by *Citrobacter freudii, Klebsiella pneumonia, Enterobactor cloacae,* and *E hafniae*. In an examination of 702 foods for faecal coilform by MPN representing ten food categories, the highest number was found in the 119 ground beef samples, with geometric mean by AOAC procedure being 59/gm. Mean number for 94 pork sausage samples was 7.9/bm. From 32 samples of minced goat meat, mean coliform count was log 2.88, mean *Enterobacteriaceae* was 3.07, while log mean was 6.57 (Andrews et al, 1979).

Biswas et al. (2011) evaluate status of microbiological contaminants in food of animal orgin. Emergence and re-emergence of disease due to pathogenic bacteria were the key of issue of the new pattern of food trades. Microbial contaminates rather common than any other form of contaminants as food animals itself harbour them hence, microbial contaminants of carcasses surface is unavoidable. The present review confirmed the importance of maintaining food process hygiene at meat packing at meat packing plants for further improvement of microbiological status of meat.

Suresh et al. (2001) conducted the investigation to provide basic information on physicochemical, functional and microbiological quality of buffalo liver. Studies indicated that liver contains higher amounts of water soluble proteins and presence of high molecular weight protein in salt soluble protein fraction.

Singh and Khan (2006) studied the microbiological analysis of buffalo meat sold at 1.5 retail shops. A total of 60 buffalo meat samples comprising of different parts of carcasses were collected from different retail shop in sterile polythenes bags during the month of hot summer (May-July, 2006). Sample immediately after collection transported to the Quality control laboratory facility of HIND AOJO Ind. Ltd, Aligarh and processed within 1-2 hour after procurement media used for different studies were obtained from Hi media, India. Total aerobic plate count (APC), total coliform *Staphylococcus aureus* fecal *Streptococci* and yeast- mold count were performed as per standard protocol of Bureace of Indian standard [Is 15478 (Part2): 2004]. Briefly, plate count agar media was used to determine total aerobic bacterial by pour plate

method, total Coliform count was performed in Tergitol-7 agar, E. coli in EMB and MA, *Staphylococcus aureus* in Baird Parker media, Glucose azide growth media for total fecal *Streptococci*, and yeast mold count was performed in yeast mold medium containing chloramphenicol. In the present study, data obtained from microbiological analysis of meat sample it was found that 64% were unsafe and unhygienic for consumption from public health point of view. The study concluded that the strict hygiene measure and cold storage facility of meat is needed to avoid any possible outbreak of food borne disease.

Sachindra and Sakhare (1996) studied the reduction in microbial load on buffalo meat by hot water dip treatment. Buffalo meat cuts from shoulder and leg portion were subjected to hot water treatment (70 and 80 ^{0} C for 30 and 60s) Meat cots dipped in water served as control. The surface sample were analyzed for microbial load, visual score for color and numerical values of color parameters ($a^{\square}$, $b^{\square}$, $L^{\square}$, w). Control samples of shoulder and leg meat had a mean total plate count (TPC) of 4.15 log CFU cm^{-2} and 3.81 log CFU cm^{-2} and *Enterobacteriaceae* counts of 2.33 log CFU cm^{-2} and 2.26 log CFU cm^{-2} respectively. Treatment of meat cuts with hot water reduced the TPC significantly (PC 0.001) with a highest reduction of 1.60 log in leg meat and 1.80 log in shoulder meat at 80^{o}C. Hot water treatment of meat eliminated *Enterobacteriaceae.* Although there was discoloration of meat by hot water treatment, the color regained during storage of meat at refrigerated temperature (4±1^{o}C). Hot water treatment of meat resulted in loss of redness ($a^{\square}$) increases in lightness ($L^{\square}$) and whiteness (w). After storage, $a^{\square}$ increased and L^{0} and w decreased. The result suggested that the dip reaction with hot water reduces the initial bacterial load. Substantially and improves the microbiological quality of buffalo meat without causing any permanent discoloration.

Thomson and Dodd (1978) studied on enrichment procedures for isolating *Salmonella* from raw meat and poultry. They found that the combined use of direct enrichment in tetrathionate broth containing green dye and pre-enrichment in buffered peptone water followed by enrichment in tetrathionate broth yielded the maximum recovery of *Salmonella* species from raw meat and poultry samples.

In an examination of 702 foods for faecal coilform by MPN representing ten food categories, the highest number was found in the 119 ground beef samples, with geometric mean by AOAC procedure being 59/gm. Mean number for 94 pork sausage samples was 7.9/bm. Form 32 samples of minced goat meat, mean coliform count was log 2.88, meant *Enterobacteriaceae* was 3.07, while log mean was 6.57. (Andrews et al. 1979)

Eliza et al. (2011) reports the identification of *Salmonella* serotypes in meat samples submitted to the Veterinary Research Institute (VRI) for diagnosis. A total of 425 *Salmonella* isolates were received from the Veterinary Public Health Laboratory and Regional Veterinary Laboratories, Malaysia from January to December 2009. All were serotyped for *Salmonella* serotypes using Kauffmann-White classification scheme. Out of the total, 31 different serotypes were identified from buffalo, beef, poultry and pork meat. The dominant serotypes identified were *S.* Typhimurium (12.7%), followed by *S. Enteritidis* (12.5%), *S.* Corvallis (11.6%), *S. Senftenberg* (11.1%) and *S.* Indiana (8.1%). Other *Salmonella* serotypes isolated included *S.* Typhi-Suis, *S. Weltevreden*, *S.* Albany, *S.* Agona and *S.* London. In poultry meat, *S.* Enteritidis (23.3%), *S.* Corvallis (21.8%), *S.* Indiana (15.9%) and *S.* Typhimurium (13.4%) were the common serotypes isolated. *Salmonella* Senftenberg (35%) was the most common *Salmonella* serotype identified in buffalo meat whereas, *Salmonella* Senftenberg (17.3%) and *S.* Agona (17.3%) were most commonly isolated from beef. *Salmonella* Typhi-Suis (51.2%) was mostly identified in pork followed by *S.* Typhimurium (34.1%), *S.* Weltevreden (7.3%) and *S.* Corvallis (4.9%). Results of the present study indicated that the *Salmonella* species were commonly found in beef, pork, buffalo and poultry meat samples from retail plants in Malaysia. Therefore, it is highly recommended to adopt proper personal and meat hygiene procedures in the meat production line to ensure that meat and meat products are safe for human consumption.

Kumar and Manujan (2006) studied the effect of water activity on the Physico-chemical, Microbiological and sensory qualities of buffalo meat sausage stored under ambient temperature. Water activity (a_w), temperature and p^H have been identified as the three 1^o factors controlling and p^H have been identified as the three 1^0 factors

controlling microbial growth. The water activity of buffalo meat sausage ($R_x$ed) was adjusted to 0.88 by addition of humectants viz, salt, sugar, ISP, HUP, Sodium lactate and subsequent heat reaction while the a_w the untreated sausage of the un$R_x$ed sausage was 0.932. Tyrosine value showed a significant increase throughout the storage periods. There was a marked but not significant decrease in the TBARS number of the treated samples. On 5^{th} day, The TBARS value of the $R_x$ed sausage exceeded the threshold limit of $2mgkg^{-1}$ resulting in the spoilage. The R_x had a significant inhibitory effect on the TVC, staphylococcal count and streptococcal count. Whereas in case of coliform and yeast mold count it had no significant effect. On the third day of storage the flavor and texture, juiciness and over acceptability score were well within the acceptable range. Whereas in the case of untreated samples there was a slime formation and off odour development on the 3^{rd} day of storage. Although the sensory score (over all acceptability) of the $R_x$ed samples were scored low ($p>0.05$), the product was acceptable unto 3^{rd} day. Storage whereas. The untreated samples spoiled after 1^{st} day of storage.

Ziauddin et al. (1993) studied the effect of freezing, thawing and frozen storage. On microbial profiles of buffalo meat. Studies were carried out on microbial profiles of buffalo meat frozen by plate freezing and blast freezing while thawed at room temperature, chill temperature in microwave oven and under running water. The results revealed a reduction in microbial count during frozen storage. Coliforms were highly sensitive, whereas *Staphylococci* and moulds were resistant to frozen storage. *Micrococci* were most predominant, followed by *Staphylococci, Pseudomonas* and bacilli; at the end of three months of storage period. Penicillium was the predominant mould during frozen storages of meat.

Murthy and Gaur (2003) Studied the effect of incorporation of tween 80 and magnesium chloride on the recovery of coliforms in VRB medium from fresh, refrigerated and frozen minced buffalo meat. They found that counts of colony forming units on VRB agar containing 0.05% (w/v) tween 80 and 0.1% (w/v) magnesium chloride were significantly higher than those on conventional VRB agar for pure culture suspensions of five isolates of Escherichia coli and one coliform isolate before and after freezing at $-18^{O}C$ for 7 days. Recoveries varied with the

isolates both before and after freezing. A mean recovery of 36% of the cells injured as a result of freezing was achieved by the modified medium. The efficiency of the medium was compared with plain VRB agar for the recovery of coliforms form naturally contaminated buffalo meat samples. The counts of presumptive coliforms showed highly significant ($p<0.01$) difference between the two media for both refrigerated and frozen samples whereas there was no significant difference observed for fresh samples.

Maharjan et al. (2006) studied the prevalence of Salmonella species in various raw meat samples of a local market in Kathmandu. A total of 123 raw meat samples (55 chicken, 37 buffalo, and 31 goats) were collected and analyzed relative to season. *Salmonella sps* was found in 11.4 % (14/123) meat samples eight samples of chicken that is 14.5% five samples of buffalo (13.5%) and one sample of goat (3.3%) were found to be positive for *S. pullorum* in 3.3% samples, *S. gallinarum* in 0.8%, *S.* Typhi in 16%, *S cholereesuis* in 0.8%, and salmonella of sub genus. I or II group in 4.9% samples. More than 80% meat samples microbiologically processed indicated coliform contamination seasonal prevalence of *Salmonella* was highest in the months of April/May surveys revealed unsatisfactory conditions of sanitation in the local meat market of Kathmandu.

Gupta et al. (2011) studied a simple and reliable duplex polymerase chain reaction (duplex-PCR) technique to identify and differentiate cattle and water buffalo DNA using primers that were tested on mitochondrial DNA (mtDNA) extracted from meat muscle samples. Different levels of autolysis were experimentally produced by putrefaction and heating the samples at various temperatures and conditions to simulate the various meat processing technology. The optimized PCR amplified 113 bp and 152 bp fragment of cyt b gene from mt DNA. This test was successful in detecting up to 1 pg adulteration in cattle-buffalo meat mixture. The test is a valuable tool for meat authentication and screening of cooked, putrefied and mixed samples of cattle and buffalo.

Chhetri et al. (2008) studied the antibiotic resistant *Escherichia coli* in meat samples from Kathmandu valley. They carried out on 39 meat samples (18 chickens, 12 buffs and 9 pork samples) with objectives to determine the occurrence of antibiotic resistant

E.coli. Meat samples were collected form Kathmandu valley selective cultures and disc-diffusion assays were performed for isolation and characterization of antibiotic resistant *E.coli*. Occurrence of *E.coli* was observed in 100% meat samples. Higher percentage (34%) of the isolated was found to be resistance to Ampicillin and 8% isolated exhibited resistance to more than one antibiotic (Cephalexin, Nalidixic acid and Gentamycin). None of the isolates were resistance to Tetracycline. The result revealed that meat sold in the Kathmandu valley may serve as an important vehicle for community wide dissemination of antibiotic-resistant *E.coli* which may represent a newly recognized group of medically significant food borne pathogens.

The study on the antibiotic resistance of 3600 strains of *Salmonella species* of animal origin. Ninety eight serotypes were identified and the most common serotypes were *S. Saintpanl*, *Salmonella* Typhimurium, and *Salmonella Dublin*. The resistance patterns to various antibiotics- Ampicillin, Streptomycin, Kanamycin, Neomycin, Framycetin, Gentamyein Chloramphenicol, Tetracycline and Colistin were studied. Out of 3624 strains, 1025 were found resistant to one or more antibiotics. 509 were resistant to one antibiotic, 218 to two, 117 to three, 92 to four, 59 to five, 23 to six, 6 to seven and 1 to eight. Resistance to tetracycline was the most (60% strains were resistant). The incidence of multiple resistances appeared to be increasing(Gledel et al, 1977).

Berchier et al. (1987) reported that the presence of *Salmonella* at slaughter time in at poultry processing plant, industrial water, scaled tank water, pre-chilled water, defeathered carcass, finished carcass, poultry meat and faeces of living birds were investigated. *Salmonella species* were not isolated from industrial water but they were isolated from all other materials. Of 9 sterotypes detected *Salmonella* Typhimurium was predominant.

Kontor and Baron (1981) reported that occurance of pathogenic *E. coli* on the surface of poultry carcasses at various stages of processing. They found that out of 315 *E. coli* isolated, 138 (43.8%) were pathogenic (67 enterititis causing and 71 other strains pathogenic to humans). They could not find correlation between the occurrence of pathogenic *E.coli* and the total number of coliforms.

2.2 Common Gram Negative Contaminants of Raw Meat

E. coli
Edwardsiella
Citrobacter
Klebsiella
Salmonella
Shigella
Enterobacter
Hafnia
Serratia
Proteus
Morganella
Providencia

2.3 Chracteristics of Salmonellae

Salmonellae are aerobic and facultative anaerobic, grow readily on sample media over a p^H range of 6-8 and temperature ranges of 15-41^{0}c (optimum 37^{0}c). Colonies are large, circular low convex and smooth. They are more translucent than coliform colonies. On Mac conkey media, colonies are colorless due to the absence of lactose fermentation. The commonly used media include selenite F broth and tetrathionate broth. They ferment gulcose, maltose and mannitol with production of acid and gas except *S.* Typhi which is an aerogenic. They do not ferment lactcose, sucrose and salicin. They are indole negative, MR positive, VP citrate negative. They are urease negative and H_2S produced, except by *S.* paratyphi A, *S.choleraesuis* and some other species.

Singh and Sharma (1998) isolated cytotoxin of *Salmonella enterica* subspecies *Enterica serovar* Weltevreden (BM-1643), from buffalo meat, was purified and characterized physicochemically and immunologically. Cell-free culture supernatant (CFCS) of the organism showing marked cytotoxicity to Vero cells and least enterotoxicity to rabbit ligated ileal loop (RLIL) model, was salt precipitated with ammonium sulphate (60% saturation level) and dialysed. Precipitated dialysed

preparation (60% PDP) when filtered through Sephadex G-100 column yielded two peaks, of which second peak (SG-100 SP) contained the cytotoxic activity. Upon filtration of SG-100 SP through SG-200 column, three peaks were obtained. Second peak (SG-200 SP), which was cytotoxic, yielded a single protein band of approximately 60-70 kDa in polyacrylamide gel electrophoresis and 3 protein bands of lower, molecular weight (13.5-56 kDa) in SDS-PAGE analysis. Cytotoxic preparation was maximally active at pH 7 to 8. On heating above 60^0C, cytotoxicity decreased gradually with insignificant activity left after treatment at 121^0C (15 min). Cytotoxin was inactivated by treatment with trypsin and protease but not by papain or lipase enzymes. It was immunogenic in rabbit and antiserum neutralized the cytotoxicity of cytotoxic preparations of homologous as well as heterologous *Salmonella* serovars.

We can separate the enteric fever group biochemically the following table:

Table 2: Biochemical character of typhoid and paratyphoid bacilli

	Gulcose	Xylose	d- Tartrate	Mucate
S. Typhi	A	d	A	D
S. Paratyphi A	AG	_	_	_
S. Paratyphi B	AG	AG	_	AG
S. Paratyphi C	AG	AG	AG	_

Pathogenicity:

Salmonellae are strict parasites of animals and humans. *S.* Typhi and *S.* Paratyphi A are confined human beings, while other *salmonellae* are parasitic in domestic animals, rodents birds and reptiles. *S. abortus-equi* is found only in hours while *S. gallinarum* in poultry *S. typhimurium* is found in animals, birds and humans *S. typhimurium* and *S. enteritidis* cause fatal septecemia in rats and mice *S. pullorum* causes 'white diarrhea' in chicks and *S. gallinarum* causes fowl typhoid. In humans, *salmonellae* cause enteric fever, septicemia, with or without local suppurative lesions, and gastroenteritis or food poisoning.

Mechanism of Pathogenesis of Enteric Fever:

The enteric fever includes typhoid fever caused by *S.* Typhi and paratyphoid fever caused by *S.* Paratyphi A, B and C. the infection is acquired by ingestion of about 10^3 to 10^6 bacilli. After reaching gut, the bacilli themselves to the ileal mucosa and penetrates to the lamina propria and submucosa. They are phagocytosed by polymorphs and macrophase and enter the mesentric lymphodes where they multiply and via the thoracic dust enter to the blood stream. They are further multiplied in liver, gall bladder, spleen, bone marrow, lymph nodes, lungs and kidneys where bacteremia occurs heralding the onset of clinical disease. It multiplies abundantly in the gall bladder and is discharged continuously into the Peyer's patches and lymphoid follicals of the ileum there occurs an inflamations of bowel leads to typhoid ulcers. Ulceration of bowel lead to intestinal perforation and hemorrhage.

The incubation period is usually 7-14 days. The clinical course may vary from mild undifferentiated pyrexia (abulent typhoid) to a rapidly fatal disease. The onset usually leads with headache, malaise, anorexia, a coated tongue and abdominal discomfort with either constipation or diarrhea. The typical features are step-ladder pyrexia, with relative bradycardia and toxemia, hepatomegaly. The collapse, bronchitis or bronchopneumonia. Some develop psychoses, deafness, or meningitis, cholecystisis, arthritis abscess, periosteitis nephritis, hemolytic anemia, venous thromboses and peripheral neuritis.

S. paratyphi A and B cause paratyphoid fever which resembles typhoid fever paratyphoid C may cause paratyphoid fever but may leads to septicemia with suppurative complications.

Treatment:

The drug that has been found effective against typhoid fever includes Chloramphenicol, Streptomycin,Ttetracycline, Ampicillin, Amoxyllin, Furazolidone and Cotrimoxazole.

2.4 Antibiotic Resistance among Raw Meat Isolates

Antibiotic, described as any substance produced by microorganism i.e. antagonistic to the growth of other antibiotic in high dilution .The extrachromosomal mechanism of drug resistant was first reported by Japanese worker. The resistance to antibiotic is due to the production of degrading enzymes. Bacteria may acquire antibiotic resistance by mutation or by one of the method of extrachromosomal gene transfer. Mutation is a random, heritable variation caused by an alteration in the nucleotide sequence of DNA of a cell. Determinant (r-factor) for each of the antibiotics. The whole plasmid (RTF+r) is known as R-factor.

Transferable antibiotic resistance is seen widely in various pathogenic and commensal bacteria of man and animals, such as *Entrobacteriaceae, Vibrio, Pseudomonas, Pasteurella*. This transfer can be affected easily in laboratory but not in normal gut as it is inhibited by several factors like anaerobic conditions, bile salts, alkaline pH and many anaerobic gram positive bacteria that minimize the chances of contact between donor cells and recipient cells. But in the intestine of persons on oral antibiotics therapy, transfers occurs easily due to the destruction of sensitive normal flora and the selection pressure produced by the antibiotic.

Mutational resistence is of two types:

(i) The stepwise mutation, as seen with penicillin, where high levels of resistence are achieved by a series of small-step mutation,and

(ii) The one step mutation, as seen with streptromycin, where the mutants differ widely in the degree of resistance.

When a person is treated with single antibiotic, the organism die but soon resistant mutant appear and multiply excessively. If two or more antibiotics are used for combined treatment mutant resistance to one drug will be destroyed by the other drug. The outcomes of a mutant exhibiting resistance to multiple drugs are not common as it do not exist.

Transferable drug resistant mediated by the R factor is the most important method of antibiotic resistance. Removal of R factor gives resistance to several antibiotics and therefore treatment with combination of antibiotic is not useful.

The resistant mutant has a lower growth rate and reduced virulence as compared to wild strains. Whereas bacteria carrying R factors are apparently normal in other respects. In some cases R factors may lead to enhanced virulence. Multiple drug resistant was initially seen in bacteria causing diarrhea and such other mild infections did not call for antibiotic treatment as a routine. But subsequently it has spread to virtually all pathogenic bacteria affecting and animals. The only way to prevent the spread of multiple resistances is to restrict the use of antibiotics.

CHAPTER-III
MATERIALS AND METHODS

3.1 Materials

All the materials, equipments, media and reagent used in this study are systematically listed in the Appendix I.

3.2 Methodology

Standard microbiological methodology was used to complete this study in the central Department of Microbiology, Kritipur Kathmandu. The study was conducted between December 2010 to May 2011. Due to time constraint, seasonal variation of micro flora in buff meat could not be studied. Altogether 73 meat samples of buff meat were studied. The samples were collected from eighteen different sampling sites Bagbazar, Tanglafat, Kirtipur, Sundhara, Balkhu, Kalimati, Thamel, Chetrapati, Kuleshwor, Chabahil, Satdobato, Basundhara, Putalisadak, Matidevi, Asan, Naxal, Tinkune, and Swoyambhu.

3.2.1 Sampling Sites

The 18 locations were Bagbazar, Tanglafat, Kirtipur, Sundhara, Balkhu, Kalimati, Thamel, Chetrapati, Kuleshwor, Chabahil, Satdobato, Basundhara, Putalisadak, Maitidevi, Asan, Naxal, Tinkune, and Swoyambhu.

Table 3: Sampling site and number of sample

SN	Sampling site	No. of sample
1	Bagbazar	4
2	Tanglafat	4
3	Kirtipur	4
4	Sundhara	4
5	Balkhu	4
6	Kalimati	4
7	Thamel	4
8	Chetrapati	4
9	Kuleshwor	4
10	Chabahil	4
11	Satdobato	4
12	Basundhara	4
13	Putalisadak	4
14	Maitidevi	4
15	Asan	4
16	Naxal	4
17	Tinkune	4
18	Swoyambu	4

3.2.2 Sample Collection

For the appropriate sample collection the sterile plastic bag were used and the samples were kept inside the sterile plastic bags without touching the sample by the hands of butcher and finally the samples were brought to the Central Department of Microbiology, Tribhuvan University, Kirtipur within 2 hours of sample collection and were processed immediately.

3.3 Processing of the Sample

3.3.1. Enumeration of Aerobic Mesophilic Count and Coliform Count

I. Grinding of Meat

Five grams of the meat samples was aseptically transferred in to a sterile mortar and grinded by sterile pestle and it was added to 45 ml of sterile buffered peptone water (BPW). This meat homogenate was itself as 10^{-1} dilution.

II. Serial dilution of homogenate

After grinding, the meat homogenate was mixed well by shaking. Then 1ml of the homogenate was pipetted and mixed into a tube containing 9 ml of BPW and carefully mixed and labeled as 10^{-2} dilution. Similarly, the dilution was carried out up to 10^{-7} dilution and labeled 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions respectively.

III. Pour plating

A. Enumeration of aerobic mesophilic bacteria (total plate count)

One ml of homogenate and dilutions of the homogenate were pipetted out and kept into each of sterile appropriately marked duplicate plates. Then sterilized total plate count agar (TPCA), cooled to 45^0C was added into each petriplates within 15 minutes of the time of original dilution. The sample dilution and agar medium were mixed thoroughly and allowed to solidify.

B. Enumeration of Coliforms Bacteria

One ml of homogenate and dilutions of the homogenate was pipetted out and kept into sterile appropriately marked duplicate plates. Sterilized violet red bile agar (VRBA), cooled to 45^{o}C, and was poured into each plate within 15 minutes of original dilution. The sample dilution and agar medium were mixed thoroughly and allowed to solidify.

Incubation of the culture

The prepared dishes containing TPCA, VRBA were incubated at 37^{0}C for 27-48 hours in an inverted position.

IV. Counting of the colonies

After 24-48 hours of incubation, the dishes containing 30-300 colonies were counted. When the dishes contained no colonies, the result was expressed as zero bacteria per gm/ml. when the dishes (dilution/ 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 CFU a number of colonies of bacteria per gm/ml.

3.3.2 Isolation of Salmonella Species

Five ml of the enriched sample of buff homogenate. Homogenate was transferred to selenite-Fborth, and incubated at 37^{0}C for 24 hours for enrichment of Salmanella sps. After 24 hours, a loopful of enriched culture from selenite-F borth was streaked on Xylose-lysive-Deoxycholate (XLD) Agar and incubated at 37^{0}C for 24 hour.

3.3.3 Isolation of the Organisms

The homogenate was streaked on Eosine Methylene blue (EMB) agar and Mac conkey (MA) agar and incubated at 37^{0}C for 24 hours. The organisms with typical colonial characters in VRBA which were used for enumeration of coliforms were picked and sub-cultured in Nutrient agar (NA) plates and Mac conkey (MA) Agar plates and were incubated at 37^{0}C for 24 hours.

3.4 Identification of the Organisms

After obtaining the pure culture, the organisms were identified by using standard microbiological techniques as described in which includes morphological appearance of the colonies, staining Bergey's manual of systematic bacteriology reactions, biochemical properties. For each set of biochemical test, both positive and negative control strains were inoculated and one uninoculted media was also incubated and examined along with the test.

3.4.1 Biochemical Tests Used for Identification of Isolated Bacteria

Following biochemical tests were carried out to identify the isolated organisms.

I. Catalase test
II. Oxidase test
III. Oxidation/Fermentation Test (O/F Test)
IV. Voges Proskauer test (VP-Test)
V. Methyl Red Test (MR-Test)
VI. Indole production Test
VII. Urease Test
VIII. Citrate Utilization test

3.5 Antibiotic Susceptibility Test

After isolation and identification of bacteria, bacterial susceptibility to antimicrobial agent was measured in vitro by utilizing the principles of agar diffusion method. Kirby Baeur Disc diffusion method. The results were interpreted according to guideline given by Clinical Laboratory Stands and Institute (CLSI)

Four to five isolated colonies of identified organisms of a similar morphology from pure culture plate were transferred into 5 ml nutrient broth and incubated at 37^0C for 4 hours. Thus, prepared inoculums were prepared with half the density of Mc Farland tube No.1, which will give evenly speeded semi-confluent growth. A sterile swab on a wooden stick was dipped into the inoculums and rotated firmly against the upper inside wall of the tube, above the fluid level, to remove excess of inoculums. The

swab was rubbed over the entire surface of the dried Mueller-Hinton agar plates and left for 5 minutes at from temperature. The selected antibiotic discs were gently placed on the inoculated plate at a distance of 15 mm form the edge of plate and 24 mm apart from each other and also from centre to centre. The plate was left at room temperature for 30 minutes for prediffusion. The agar plate was the inverted and inoculated at 37^0C for 24 hours. The diameter of the zone of attribution of bacterial growth around each disc was measured and susceptibility or resistance to the agent in each disc was determined from a standardized table.

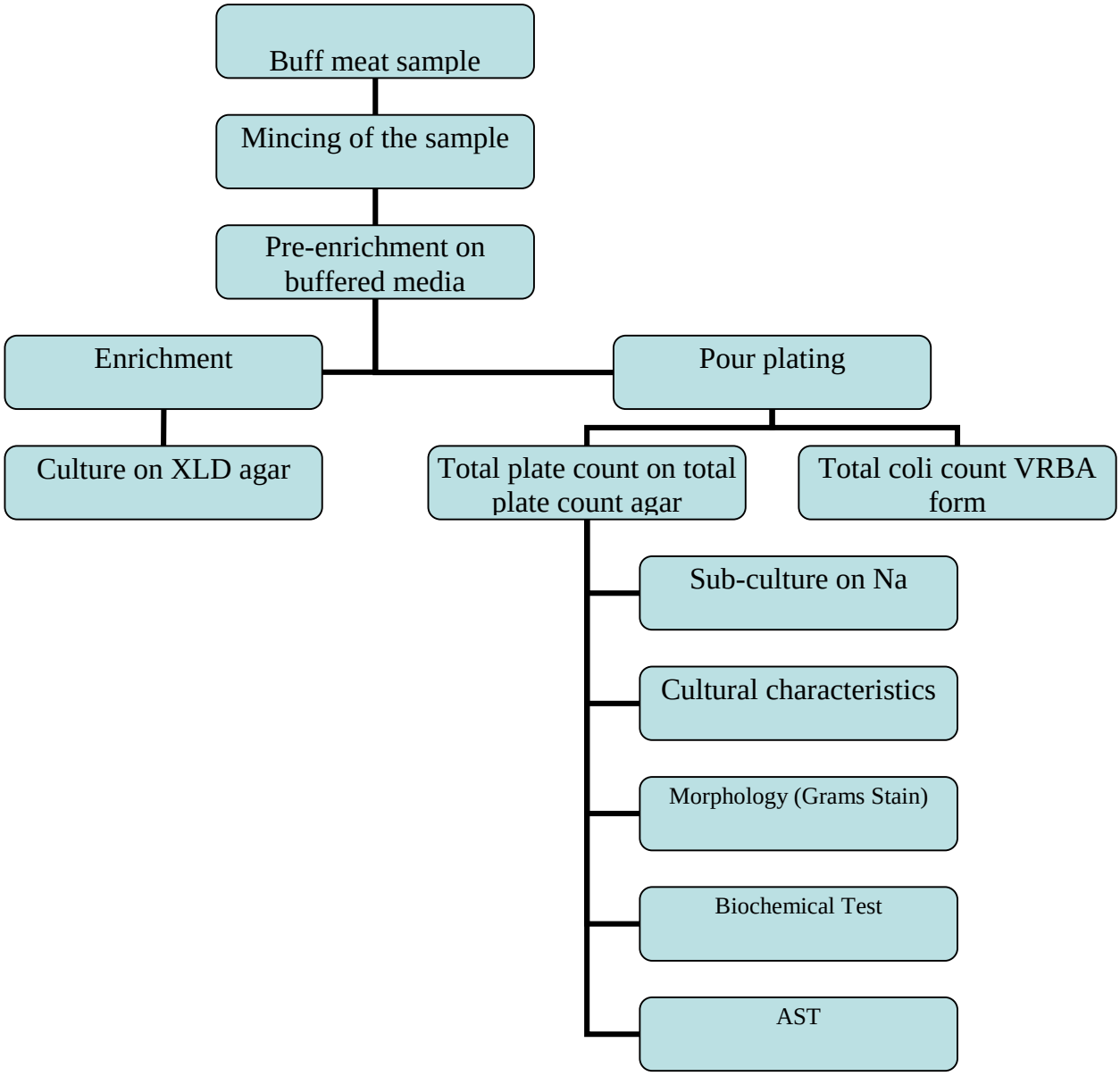
3.6 Quality Control

Every experimental setup were controlled in the respective term of quality. It includes incubator, autoclaves, microbiological media etc.

For antibiotics susceptibility testing, antibiotics disc quality was evaluated using reference strains *Escherchia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923

3.7 Data Entry and Analysis

The raw data obtained were entered in MS Excel software program. The data were tabulated and graphs were plotted. The statistical application was done whenever applicable.



Flow diagram showing sampling and analysis of raw meat sample

□

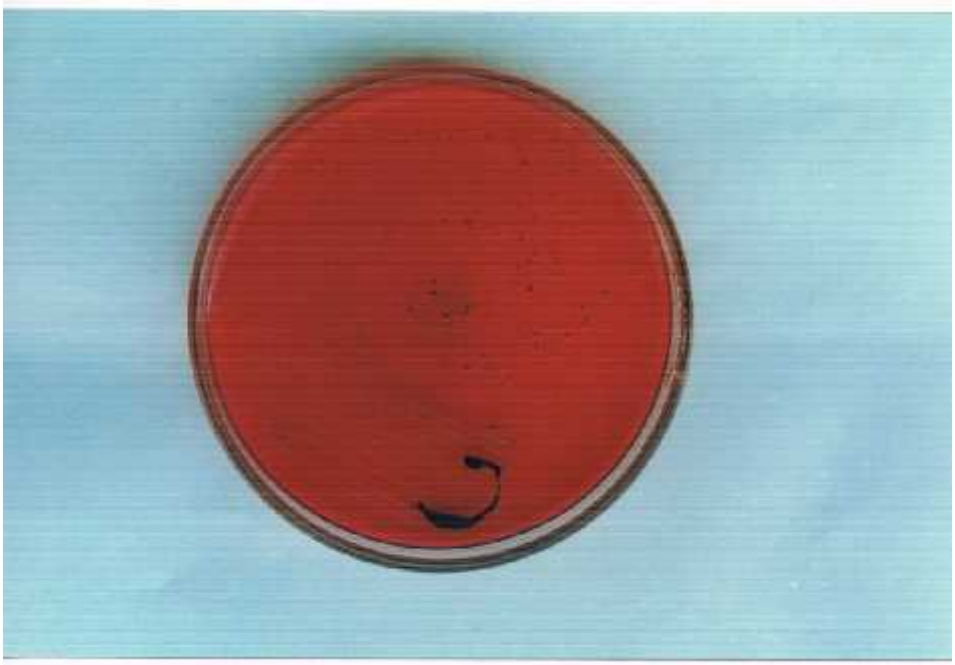


Photo 1: Red colour colony with black center XLD Agar

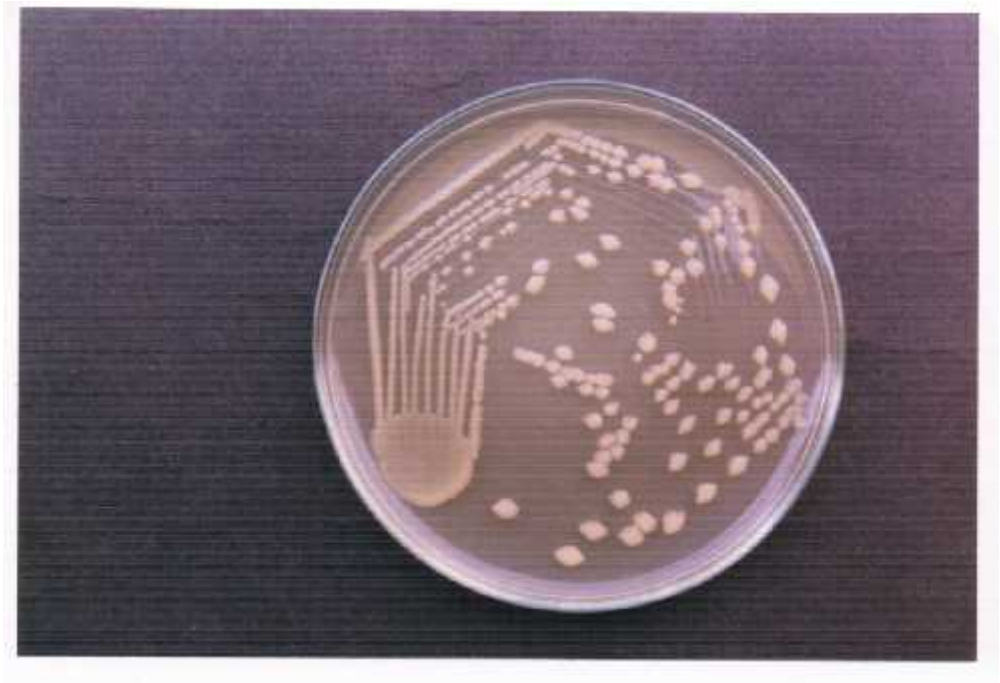


Photo 2: *Salmonella spp* on Nutrient Agar.

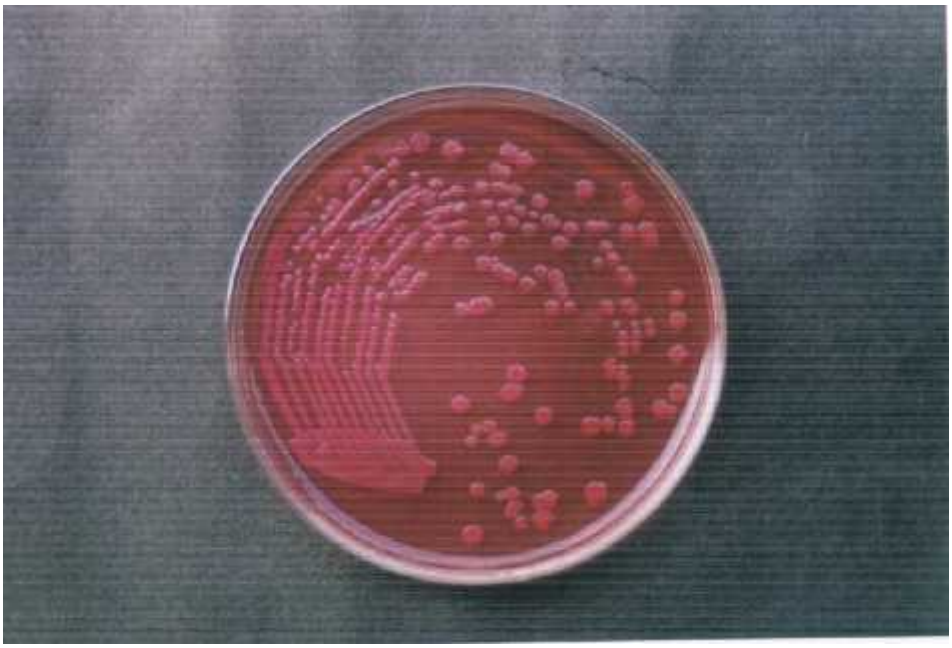


Photo 3: *E. coli* on MA.

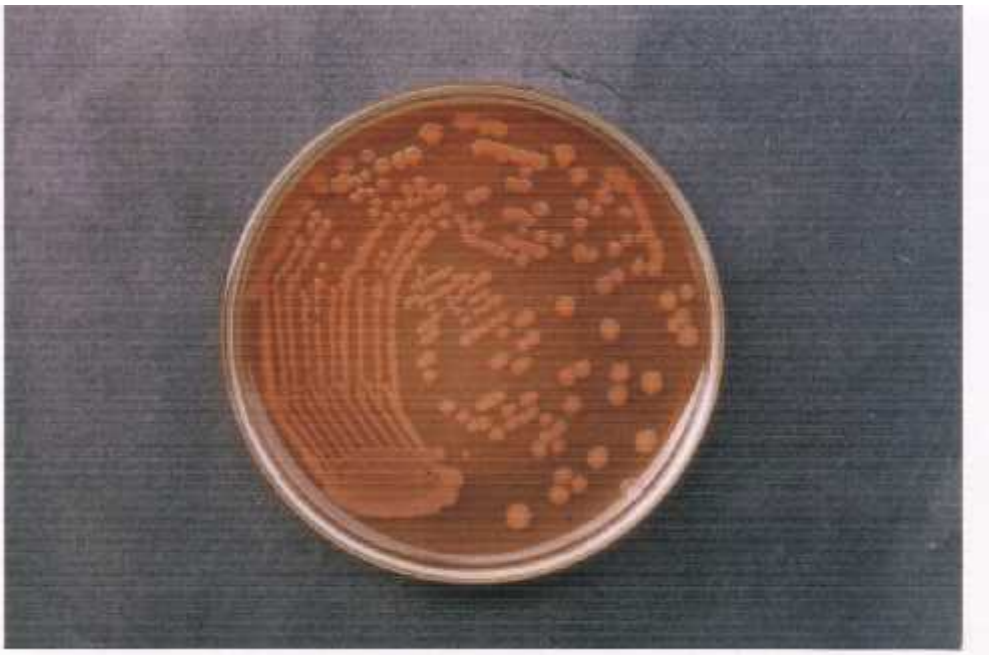


Photo 4: *Salmonella spp* on Mac Conkey Agar.

Photo 5. Biochemical test *E. coli*

Photo 6: Biochemical test of *Klebsiella oxytoca*

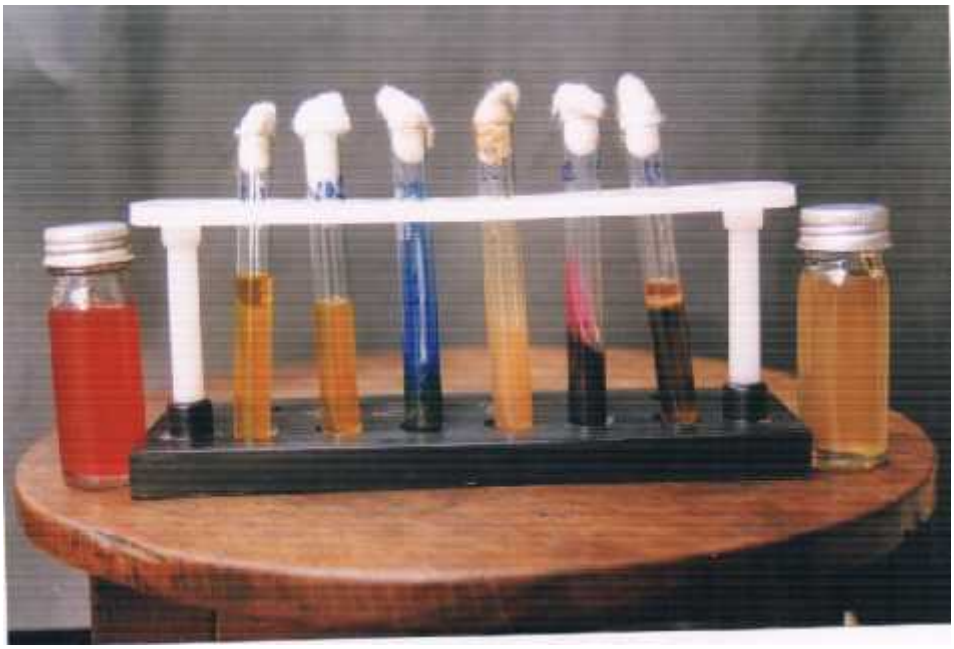


Photo 7: Biochemical test

CHAPTER –IV

RESULTS

Raw buff meat samples were collected from 18 different places of Kathmandu valley. For bacteriological analysis and their susceptibility to different antimicrobial agents sampling was done randomly. After grinding, the samples were cultured on violet Red Bile Agar (VRBA) and Plate Count Agar (PCA) by pour plate technique for total coliform counts and for total viable bacterial count respectively and with special interest on *Salmonella* species.

Table 4: Total Plate Counts and Total Coliform Counts of different samples and locations of buff meat

S.No.	Sample	Location	Total Plate Count	Total Coliform Count(cfu/gm)
1.	Ba_1	Baghbazar	1.7×10^6	1.3×10^5
2.	Ba_2		1.8×10^6	2.4×10^5
3.	Ba_3		1.7×10^6	2×10^5
4.	Ba_4		2.6×10^6	1.7×10^5
5.	Ta_5	Tanglafat	6.6×10^6	4×10^6
6.	Ta_6		6.1×10^6	6.2×10^5
7.	Ta_7		5.4×10^6	4.5×10^5
8.	Ta_8		4.9×10^6	6×10^5
9.	Ki_9	Kirtipur	4.0×10^5	4×10^5
10.	Ki_{10}		4.7×10^5	2×10^6
11	Ki_{11}		3.8×10^6	5×10^5
12	Ki_{12}		3.2×10^5	3×10^4
13	Su_{13}	Sundhara	3.5×10^6	1.8×10^5
14	Su_{14}		3.1×10^6	1.1×10^5
15	Su_{51}		1.7×10^6	4.1×10^5
16	Su_{16}		4.4×10^7	2.9×10^6
17	Bal_{17}	Balkhu	3.3×10^5	1×10^5
18	Bal_{18}		2.5×10^5	2×10^5
19	Bal_{19}		2.1×10^6	1×10^4
20	Bal_{20}		3.9×10^5	3.6×10^5
21	Ka_{21}	Kalimati	1.8×10^6	7×10^5
22	Ka_{22}		2.3×10^6	3×10^5

23	Ka_{23}		TM TC	7×10^6
24	Ka_{24}		3×10^6	4.8×10^5
25	Th_{25}	Thamel	6.1×10^6	1×10^5
26	Th_{26}		4.3×10^6	1.6×10^5
27	Th_{27}		5.6×10^6	5.6×10^5
28	Th_{28}		4.1×10^6	2.1×10^5
29	Ch_{29}	Chetrrpati	5.6×10^5	1×10^4
30	Ch_{30}		2.8×10^6	2.1×10^5
31	Ch_{31}		2.9×10^6	2.0×10^5
32	Ch_{32}		3.2×10^5	3×10^4
33	Ku_{33}	Kuleshwor	2.5×10^6	1×10^5
34	Ku_{34}		4.0×10^5	2.9×10^5
35	Ku_{35}		2.9×10^6	2.5×10^5
36	Ku_{36}		3.4×10^6	<30
37	Cha_{37}	Chabahil	3.8×10^6	5×10^5
38	Cha_{38}		8.3×10^7	7.5×10^6
39	Cha_{39}		TM TC	7.2×10^6
40	Cha_{40}		7.1×10^6	6×10^5
41	Sa_{41}	Satdobato	TM TC	6.3×10^6
42	Sa_{42}		TM TC	7×10^6
43	Sa_{43}		3.9×10^6	5×10^5
44	Sa_{44}		4.8×10^7	8×10^5
45	Bas_{45}	Basundhara	1.3×10^5	1×10^6
46	Bas_{46}		4.2×10^5	1.1×10^4
47	Bas_{47}		4.4×10^5	2.6×10^5
48	Bas_{48}		3.2×10^5	1.9×10^5
49	Pu_{49}	Putalisadak	2.6×10^6	9×10^5
50	Pu_{50}		2×10^7	8.9×10^6
51	Pu_{51}		3.1×10^6	9.6×10^5
52	Pu_{52}		2.8×10^6	9.8×10^5
53	Ma_{53}	Maitidevi	1.9×10^6	1.9×10^5
54	Ma_{54}		1.5×10^6	1.1×10^5
55	Ma_{55}		2.1×10^6	2.3×10^5
56	Ma_{56}		3.3×10^6	1.7×10^5
57	As_{57}	Asan	8.6×10^6	5×10^6
58	As_{58}		7×10^6	5.6×10^5
59	As_{59}		8.9×10^6	5.1×10^5
60	As_{60}		9.3×10^6	6.9×10^5
61	Na_{61}	Naxal	3.1×10^5	1.1×10^5

62	Na_{62}		3.0×10^5	1.5×10^5
63	Na_{63}		4.1×10^5	1.9×10^4
64	Na_{64}		4.7×10^5	2.3×10^5
65	Ti_{65}	Tinkune	TM TC	8.3×10^5
66	Ti_{66}		9.2×10^6	8×10^6
67	Ti_{67}		9.1×10^6	8.6×10^5
68	Ti_{68}		8.7×10^7	8.9×10^5
69	Sw_{69}	Swoyambhu	1.1×10^6	2.1×10^5
70	Sw_{70}		2.3×10^6	2.5×10^5
71	Sw_{71}		3×10^6	1.6×10^4
72	Sw_{72}		3.7×10^5	2.9×10^5

*TMTC – Too Many To Count

Total viable bacterial count of Buff Meat

The total plate count of buff meat of Baghbazar for Ba, was found to be 1.7×10^6 cfu/gm, for Ba_2 was found to be 1.8×10^6 cfu/gm, similarly for Ba_3 was 1.7×10^6 cfu/gm, and for Ba_4 was 2.6×10^5 cfu/gm.

Similarly the total plate count of buff meat of Tanglafat for Ta_5 found to be 6.6×10^6 cfu/gm, for Ta_6 was found to be 6.1×10^5 cfu/gm, for Ta_7 was 5.4×10^6 cfu/gm. And for Ta_8 was 4.9×10^6 cfu/gm.

The total plate count of buff meat of Kirtipur for Ki_9 was found to be 4×10^5 cfu/gm, for Ki_{10} was found to be 4.7×10^5 cfu/gm, for Ki11 was found to be 3.8×10^6 cfu/gm and for Ki_{12} was 3.2×10^5 cfu/gm.

The total plate count of buff meat of Sundhara for Su_{13} was found to be 3.5×10^6 cfu/gm, for Su_{14}, was found to be 3.1×10^6 cfu/gm, for Su_{15} was 1.7×10^6 cfu/gm and for Su_{16} was 4.4×10^7 cfu/gm.

The total plate count of buff meat of Balkhu for Ba was found to be 3.3×10^5 cfu/gm, for Ba_{18} was 2.5×10^5 cfu/gm and for Ba_{19} was 2.1×10^5 and for Ba_{20} was 3.9×10^5 cfu/gm.

The total plate count of buff meat of Kalimati for Ka_{21} was found to be 1.8×10^6 cfu/gm, for Ka_{22} was 2.3×10^6 cfu/gm, for Ka_{23} was found to be TMTC and for Ka_{24} was 3×10^6 cfu/gm.

The total plate count of buff meat of Thamel for Th_{25} was found to be 6.1×10^6 cfu/gm and for Th_{26} was found to be 4.3×10^6 cfu/gm and for Th_{27} was found to be 4.1×10^6 cfu/gm.

The total plate count of buff meat of Chhetrapati for Ch_{29} was found to be 5.6×10^5 cfu/gm, for Ch_{30} was for 2.8×10^6 cfu/gm, for Ch_{31} was 2.9×10^6 cfu/gm, and for Ch_{32} was 3.2×10^5 cfu/gm.

The total plate count of buff meat of Kuleshwor for Ku_{33} found to be 2.5×10^6 cfu/gm, for Ku_{34} was 4.0×10^5 cfu/gm, for Ku_{35} was 2.9×10^6 cfu/gm and for Ku_{36} was 3.4×10^6 cfu/gm.

The total plate count of buff meat of Chabahil for Ch_{37} was found to be 3.8×10^6 cfu/gm, for Ch_{38} was found to be 8.3×10^7 cfu/gm, for Ch_{35} was TMTC and for Ch_{40} was 7.1×10^6 cfu/gm.

The total plate count of buff meat of Satdobato for Sa_{41} was TMTC for Sa_{42} was found to be TMTC for Sa_{43} was found to be 3.9×10^6 cfi/gm and for Sa_{44} was found to be 4.8×10^7 cfu/gm.

The total plate count of buff meat of Basundhara for Ba_{45} was found to be 1.3×10^5 cfu/gm, for Ba46 was 4.2×10^5 cfu/gm, for Ba_{47} was 4.4×10^5 cfu/gm and for Ba_{48} was 3.2×10^5 cfu/gm.

The total plate count of buff meat of Putalisadak for Pu_{49} was 2.6×10^6 cfu/gm, fro Pu_{50} was 2×10^7 cfu/gm, for Pu_{51} was 3.1×10^6 cfu/gm and for Pu_{52} were 2.8×10^6 cfu/gm.

The total plate count of buff meat of Maitidevi for Ma_{53} was found to be 1.9×10^6 cfu/gm, for ma_{54} was 1.5×10^6 cfu/gm, for Ma_{55} was 2.1×10^6 cfu/gm and for ma_{56} 3.3×10^6 cfu/gm.

The total plate count of buff meat of Asan for As_{57} was found to be 8.6×10^6 cfu/gm, for As_{58} was 7×10^6 cfu/gm, for As_{59} was 8.9×10^6 cfu/gm and for As_{60} was 9.3×10^6 cfu/gm.

The total plate count of buff meat of Naxal for Na_{61} was fouind to be 3.1×10^5 cfu/gm, for Na_{62} was 3×10^5 cfu/gm, for Na_{63} was 4.1×10^5 cfu/gm and for Na_{64} was 4.7×10^5 cfu/gm.

The total plate count of buff meat of Tinkune for Ti_{65} was found to be TMTC, for Ti_{66} was 9.2×10^6 cfu/gm, for Ti_{67} was 9.1×10^6 cfu/gm and for Ti_{68} was 8.7×10^7 cfu/gm

Lastly, the total plate count of buff meat of Swoyambhu for Sw_{69} was found to be 1.1×10^6 cfu/gm for Sw_{70} was 2.3×10^6 cfu/gm, for Sw_{71} was 3×10^6 cfu/gm and for Sw_{73} was 3.7×10^5 cfu/gm

Total coliform count of Buff Meat

The total coliform count of buff meat of Baghbazar for Ba1 showed to be 1.3×10^5 cfu/gm, for Ba_2 showed to be 2.4×10^5 cfu/gm, for Ba_3 showed 2×10^5 cfu/gm and Ba_4 showed 1.7×10^5 cfu/gm.

The total coliform count of buff meat of Tanglafat for Ta5 showed to be 4×10^6 cfu/gm, for Ta6 showed 6.2×10^5 cfu/gm, for Ta7 showed to be 4.5×10^5 cfu/gm and for Ta_8 showed 6×10^5 cfu/gm.

The total coliform count of buff meat of Kirtipur for K_{i9} showed to be 4×10^5 sfu/gm, for Ki_{10} showed 2×10^6 cfu/gm, for Ki_{11} showed 8×10^5 cfu/gm and for Ki_{12} showed 3×10^4 cfu/gm.

The total coliform count of buff meat of Sundhara for Su_{13} showed 1.8×10^5 cfu/gm, for Su_{14} showed 1.1×10^5 cfu/gm, for Su_{15} showed 4.1×10^5 cfu/gm and for Su_{16} showed 2.9×10^6 cfu/gm.

The total coliform count of buff meat of Balkhu for Ba_{17} showed 1×10^5 cfu/gm, for Ba_{18} showed to be 2×10^5 cfu/gm, for Ba_{19} showed 1×10^4 cfu/gm and for Ba_{20} the total colifarm count were 3.6×10^5 cfu/gm

The total coliform count of buff meat of Kalimati for Ka_{21} showed to be 7×10^5 cfu/gm, for Ka_{22} showed to be 3×10^5 cfu/gm, Ka_{23} showed as 7×10^6 cfu/gm, and for Ka_{24} showed 4.8×10^5 cfu/gm.

The total coliform count buff meat of Thamel for Th_{25} showed $1x10^5$ cfu/gm for Th_{26} showed to be $1.6x10^5$ cfu/gm , for Th_{27} showed 5.6×10^5 cfu/gm and Th_{28} showed $2.1x10^5$ cfu/gm.

The total coliform count of buff meat of chetrapati for Ch_{29} showed to be $1x10^5$ cfu/gm, for Th_{30} showed $2.9x10^5$ cfu/gm for Ch_{31} showed $2.0x10^5$ cfu/gm and for Ch_{32} showed $3x10^4$ cfu/gm.

The total coliform count of buff meat of kuleshwor ku_{33} showed do be 1×10^5 cfu/gm cfu/gm, for ku_{34} showed $2.9x10^5$ cfu/gm, for ku_{35} showed $2.5x10^5$ cfu/gm and finally for Ku_{36} showed <30 cfu/gm.

The total coliform count of buff meat of chabahil for ch_{37} showed to be $5x10^5$ cfu/gm, for ch_{38} showed to be $7.5x10^6$ cfu/gm, for ch39 showed to be $7.2x10^6$ cfu/gm and Ch_{40} showed to be $6x10^5$ cfu/gm.

The total coliform count of buff meat of satdobato for sa_{41} showed to be $6.3x10^6$ cfu/gm fro Sa_{42} showed 7×10^6 cfu/gm, for Sa_{43} showed 5×10^5 cfu/gm, and for Sa_{44} showed $8x10^5$ cfu/gm.

The total coliform count of buff meat of Basundhara for Ba_{45} showed to be $1x10^6$ cfu/gm for Ba_{46} showed $1.1x10^4$ cfu/gm, for Ba_{47} showed $2.6x10^5$ cfu/gm and for Ba_{48} showed $1.9x10^5$ cfu/gm.

The total coliform count of buff meat of putalishadak for Pu_{49} showed to be $9x10^5$ cfu/gm, for Pu_{50} showed $8.9x10^6$ cfu/gm, for Pu_{51} showed $9.6x10^5$ cfu/gm, for Pu_{52} showed $9.8x10^5$ cfu/gm.

The total coliform count of buff meat of Maitidevi for Ma_{53} showed $1.9x10^5$ cfu/gm, for Ma_{54} showed $1.1x10^5$ cfu/gm, for Ma_{55} showed to be $2.3x10^5$ cfu/gm and for Ma_{56} showed $1.7x10^5$ cfu/gm.

The total coliform count of buff meat of Asan for As_{57} showed $5x10^6$ cfu/gm, for As_{58} showed $5.6x10^5$ cfu/gm, for As_{59} showed $5.1x10^5$ cfu/gm and for As_{60} showed $6.9x10^5$ cfu/gm.

The total coliform count of buff meat of Naxal for Na_{61} showed $1.1x10^5$ cfu/gm for Na_{62} showed $1.5x10^5$ cfu/gm, for Na_{63} showed $1.9x10^4$ cfu/gm and for Na_{64} showed $2.3x10^5$ cfu/gm

The total coliform count of buff meat of Tinkune for Ti_{65} showed to be $8.3x10^5$ cfu/gm, for Ti_{66} showed $8x10^6$ cfu/gm, for Ti_{67} showed $8.6x10^5$ cfu/gm and for Ti_{68} showed $8.9x10^5$ cfu/gm.

The total coliform count of buff meat of Swoyambhu showed to be $2.1x10^5$ cfu/gm, for Sw_{70} showed to be $2.5x10^5$ cfu/gm, for Sw_{71} showed $1.6x10^4$ cfu/gm, and for Sw_{72} showed $2.9x10^5$ cfu/gm.

Table 5: Types of bacterial isolates from different locations

S.N.	Location	Types of organism isolated
1.	Baghbazar	*E.coli* *Klebsiella oxytoca* *Enterobacter species*
2.	Tanglafat	*E.coli* *K. pneumonia*
3.	Kirtipur	*E.coli* *Citrobacter diversus*
4.	Sundhara	*E.coli* *Citrobacter diversus* *Salmonella species* *Klebsiella oxytoca*
5.	Balkhu	*E.coli* *Citrobactes freundii*
6.	Kalimati	*E.coli* *Citrobacter diversus* *Enterobacter species*
7.	Thamel	*E.coli* *Klebsiellsa oxytoca* *Salmonells species*

8.	Chetrapati	*E.coli* *Citrobacter freundii*
9.	Kuleshwor	*E.coli*
10.	Chabahil	*E.coli* *Salmonella species* *K. pneumonia*
11.	Satdobato	*E.coli* *Salmonella species* *Enterobacter species* *Citrobacter diversus*
12.	Basundhara	*E.coli*
13.	Putalisadak	*E.coli* *Citrobacter freundii*
14.	Maitidevi	*E.coli* *Enterobacter species*
15.	Ason	*E.coli* *K. oxytoca*
16.	Naxal	*E.coli*
17.	Tinkune	*E.coli* *Citrobacter diversus* *Klebsiella oxytoca* *S.Typhi*
18.	Swoyambhu	*E.coli* *Citrobacter diversus*

Table 6: Frequency of gram negative isolates

SN	Gram negative isolates	Frequency
1	*E.coli*	18
2	*Citrobacter diversus*	6
3	*Klebsiella oxytoca*	5
4	*Klebsiella pneumonia*	2
5	*Enterobacter sps*	4
6	*Citrobacter freundii*	3
7	*Salmonella sps.*	4
8	S. Typhi	1

Antibiotic susceptibility patterns of the bacterial isolates from meat

The antibiotic sensitivity test of the isolates were Amikacin, Cotrimoxazole, Chloramphenicol, Ofloxacin, Nalidixic Acid, Tetracycline

Fig.1: Antibiotic susceptibility patterns of *E.coli* isolated

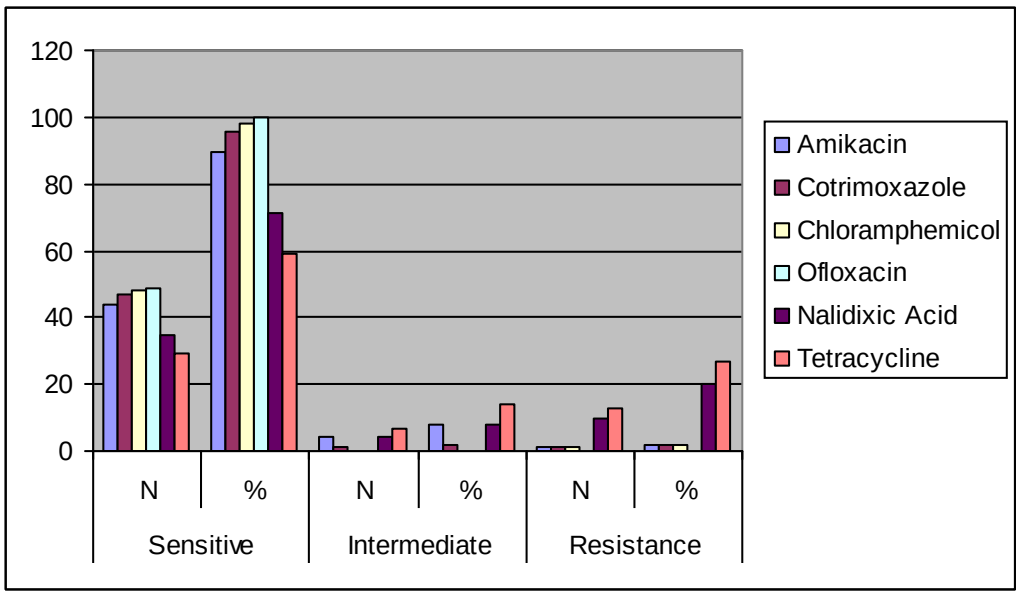


Fig.2: Antibiotic susceptibility patterns of *Klebsiella oxytoca*

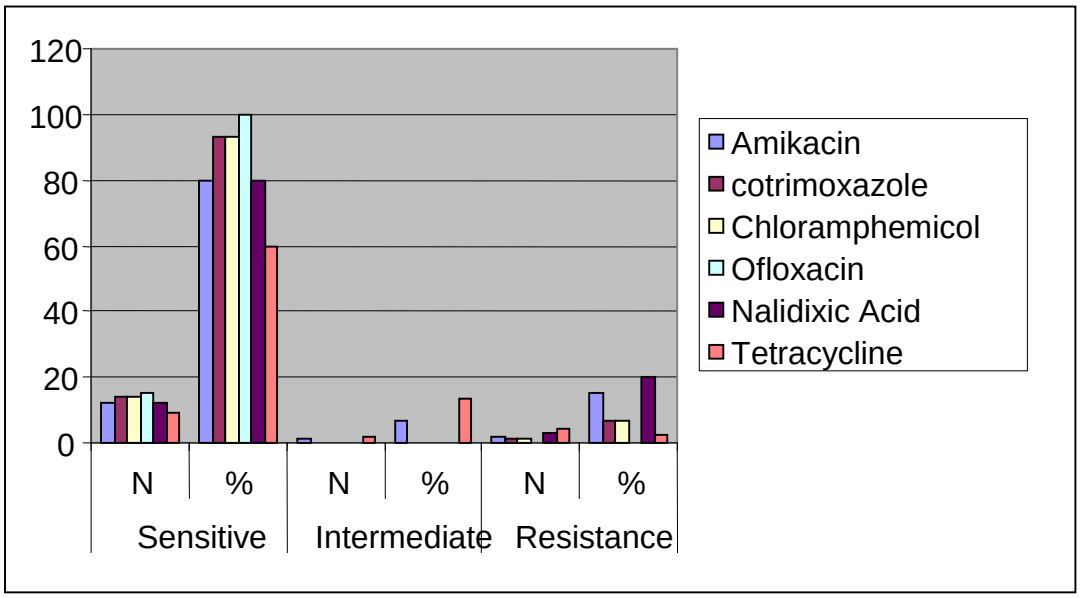


Fig. 3: Antibiotic susceptibility patterns of *Klebsiella pneumoniae*

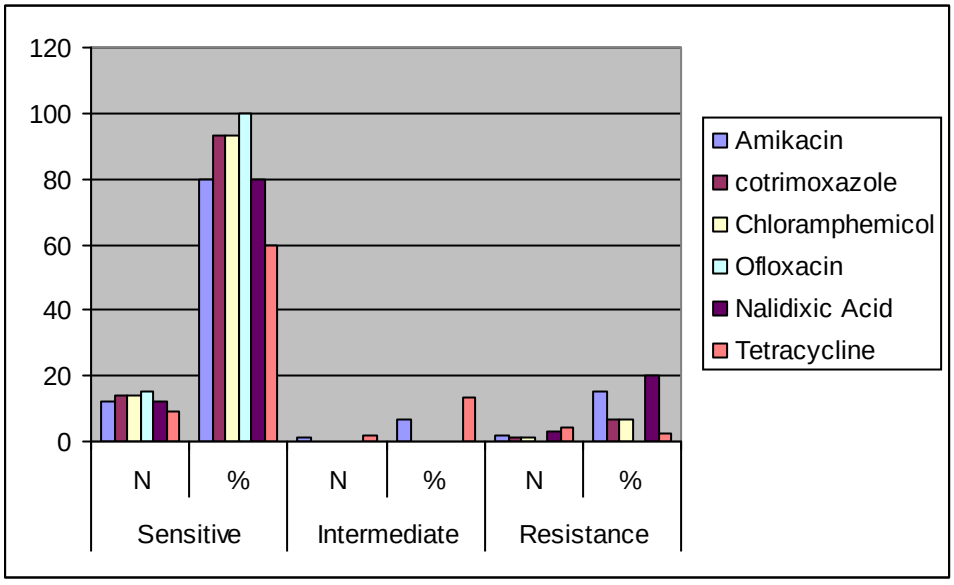


Fig. 4: Antibiotic susceptibility patterns of *Enterobacter spp*

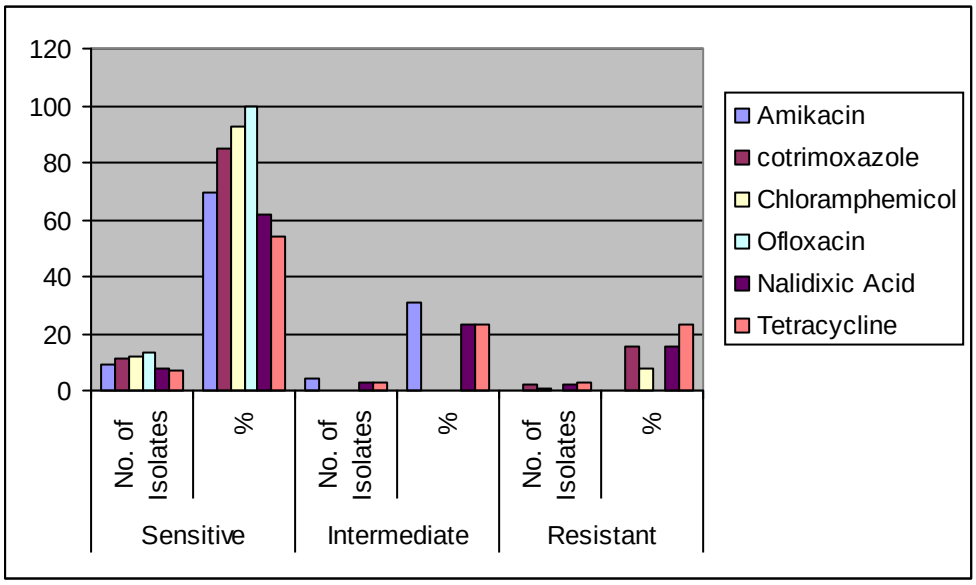


Table 7: Antibiotic susceptibility patterns of *Citrobacter diversus*

Antibiotics	**Sensitive**		**Intermediate**		**Resistant**	
	No. of Isolates	%	No. of Isolates	%	No. of Isolates	%
Amikacin	14	100	0	0	0	0
cotrimoxazole	14	100	0	0	0	0
Chloramphenicol	14	100	0	0	0	0
Ofloxacin	14	100	0	0	0	0
Nalidixic Acid	7	50	3	21.4	4	28.6
Tetracycline	10	71	1	7.2	3	21.4

Table 8: Antibiotic susceptibility patterns of *Citrobactes fruendii*

Antibiotics	Sensitive		Intermediate		Resistant	
	No. of Isolates	%	No. of Isolates	%	No. of Isolates	%
Amikacin	12	92.3	1	7.7	0	0
Cotrimoxazole	10	76.9	1	7.7	2	15.4
Chloramphenicol	13	100	0	0	0	0
Ofloxacin	13	100	0	0	0	0
Nalidixic Acid	9	69.2	3	23.1	1	7.7
Tetracycline	9	69.2	3	23.1	1	7.7

Table 9: Antibiotic susceptibility patterns of *Salmonella spp*

	Sensitive		Intermediate		Resistant	
	No. of Isolates	%	No. of Isolates	%	No. of Isolates	%
Cotrimoxazole	2	100	0	0	0	0
Chloramphenicol	2	100	0	0	0	0
Ofloxacin	2	100	0	0	0	0
Nalidixic Acid	2	100	0	0	0	0
Tetracycline	1	50	1	50	0	0
Amikacin	1	50	0	0	1	50

Table 10: Antibiotic susceptibility patterns of *Salmonella* Typhi

	Sensitive		**Intermediate**		**Resistant**	
	No. of Isolates	%	No. of Isolates	%	No. of Isolates	%
Cotrimoxazole	5	100	0	0	0	0
Chloramphenicol	5	100	0	0	0	0
Ofloxacin	5	100	0	0	0	0
Nalidixic Acid	3	60	1	20	1	20
Tetracycline	4	80	0	0	1	20
Amikacin	2	40	1	20	2	40

CHAPTER V
DISCUSSION

The number of organism present in the meat and meat products at any given time depends on its handling, storage time and temperature. Microorganisms including many pathogens favor the meat and meat products due to their high nutritive value. The increasing population, urbanization, modernization and industrialization of the Kathmandu valley is also responsible for the pollution. The impact in pollution is also on various foods borne disease due to contribution by various pathogenic bacteria.

Microorganisms set into the meat and meat products by water, unclean utensils, knives, unscientific slaughtering practices and handling methods. Besides, the environmental contamination and the handling of meat in its preparation and sales. Due to lack of scientific method of storage and due to lack of knowledge of microorganisms, many types of microorganisms introduce into the meat. Once microorganisms introduced into meat, they multiply rapidly and reach levels sufficient to produce infection or intoxications depending upon the types of in microorganisms.

The contaminating organism may include microorganisms responsible for food borne illness. But the number or dose of organisms necessary to infect or to produce sufficient toxin to cause symptoms not only varies with the different microorganisms but also varies with the resistance or some other condition of the person who utilized the meat and meat products. Even though the microbial population in the meat and the microbial contamination is an indicator or poor sanitary practice in the preparation and storage of the food. The availability of clean water was one of the most important problems faced by the butchers.

The survey showed that the shops used water from different sources such as river, tap, well and stone sprouts according to the availability in a near establishment. The storage of water was done in bucket on reserved tank and their cleaning process depended on the size of the storage vessel. Not only sanitary facilities, the survey results should that’s the knowledge of sanitation among the butchers and sellers of the traditional shops were comparatively limited. Meat was not protected during storage.

Most of them were kept in refrigerator. Hence, the threats of contaminating meat with organisms like *E.coli*, *Salmonella* species, other coliforms increases which itself raises an important concern or safety of meat and meat products.

Although 72 sample of raw buff meat were collected. Efforts were made to isolate the contaminated bacteria and their susceptibility to different antimicrobial agent i.e. antibiotic susceptibility test.

In this study, altogether 72 buff meat samples from 18 different locations were randomly analyzed. The viable bacterial count ranges from $8.7x10^7$ to $1.1x10^5$ cfu/gm. The highest number of microorganism's i.e. TMTC was reported from Kalimati, Chabahil, satdobato and Tinkune where as the lowest number of microorganisms were reported from Swoyambhu.

Similarly, the total coliform counts were done from the 72 buff meat samples from 18 different locations randomly. The total clliform counts ranges from $8.9x10^6$ cfu/gm to <30 cfu. The highest total coliform counts were found in the sample from Putalishadak where as the lowest total coliform counts were reported from Kuleshwor.

From this study, the Kalimati, Chabaihil, Satdobato, Tinkune and Putalisadak sample was found to containing the hightest in total viable bacterial count and total coliform count. This may be due to during sampling the meat was found to be sold along with the animal gut as well as there were not any sanitation facilities like the slaughtering were done on river banks and may be that the meat was sold by spreading on the floor. The other reasons may be due to Kalimati, Chabaihil, Satdobato, Tinkune and Putalisadak is highly polluted areas because of its vegetable market, high traffic and high human activities.

From this study, the lowest total viable bacterial count and total coliform count were reported from swoyambhu and kuleshwor which may be due to less crowded areas, proper sanitary condition or the samples were kept in refrigerator or due to proper washing.

Altogether 5 different types of bacteria were isolated from 72 samples from 18 different locations of Kathmandu valley. Among the 122 isolates, *E.coli* was found to be predominant organism followed by *Klebsiella oxytoca, Citrobacter diversus, Enterobacter sps, Citrobacter, Freundii, Klebsiella pneumoniae, salmonella spp.*

The sample from Baghbazar was contaminated from *E.coli, Klebsiella Oxytoca, Enterobacter Spp*. The sample of tanglafat was contaminated with *Ecoli, K. Pneumoniae.* The sample from Kirtipur was contaminated for *E.coli, Citrobacter diversus*. The sample from Sundhara was contaminated from *E.coli, Citrobacter diversus, Salmonella spp, Klebsiella oxytoca.* The sample from Balkhu was contaminated from *E.coli, Citrobacter freundii*. The sample from Kalimati was contaminated with *E.coli, Citrobacter diversus, Enterobacter spp* and from Thamel was contaminated with *E.coli, Klebsiella oxytoca, Salomonella spp*. The sample from Chetrapati was contaminated with *E.coli, C. freundii*. The sample from Kuleshwor was contaminated with *E.Coli* and from Chabahil was contaminated with *E.coli, Salmonella spp, K.pneumomiae*. The sample from Satdobato was contaminated with *E.coli, salmonella spp, Enterobacter spp*. *Cirtrobacter diversus*. The sample from Basundhara was contaminated with *E.coli* Similarly that of Putalishadak was contaminated with *E.coli, Citrobacter freundii*. The sample from Maitidevi was contaminated with *E.coli, Enterobacter spp* and that from Asan was contaminated with *E.coli, K. oxytoca*.

The sample from Naxal was contaminated with *E.coli* and that of Tinkune with *E.coli, Citrobacter diversus, Enterobacter spp* and lastly sample from Swoyambhu was contaminated with *E.coli, Cirtrobacter diversus*.

Total 6 different types of antibiotic disc were used for the antibiotic susceptibility test 122 isolated species of bacteria were tested for all 6 antibiotics.

All the isolates were found to be sensitive to antibiotic like Amikacin, Cotrimoxazole, borad spectrum antibiotic like Chloramphenicol, fluoroquinolones such as ofloxacin, Nalidixic Acid, Tetracycline no. organisms were found to be resistant to ofloxacin except *Salmonella* Typhi.

Among the 49 isolates of *E.coli* which were tested against 6 different antibiotics, it was found that *E.coli* was 100% sensitive to ofloxacin. Out of which, 48 isolates 97.9% were sensitive against chloramphenicol, 95.9% were sensitive to cotrimoxazole, 89.8% were sensitive to Amikacin, 71.4% were sensitive with Nalidixic Acid, 59.2 % to tetracycline.

Fifteen different isolates of *Klebsiella oxytoca* were tested against 6 different antibiotics. All the isolates i.e 100% were sensitive against ofloxacin whereas 93.3% were sensitive against contrimoxazole and chloramphenicol, 12% were sensitive against Amikacin and Nalidixic Acid, 9 % were sensitive against tetracycline.

Similarly, antibiotic sensitive test was done with 11 isolates of *K. pneumoniae* which was tested with 6 different antibiotics. From the study it was found that 100% isolates were sensitive against chloramphenicol and ofloxacin, 90.9% were sensitive to cotriomoxazole, 81.8% sensitive to Nalidixic Acid, 72.7% sensitive to Amikacin, and 54.5% sensitive to Tetraclydine.

From this study, 13 isolates of *Enterobacter spp.* Were tested against 6 different antibiotics 100 % isolates were sensitive to ofloxacin 92.3% were sensitive against chloramphenicol. 84.6 % were sensitive against Cotrimoxazole, 69.2% were sensitive against Amikacin 61.5% sensitive against Nalidixic Acid and 53.8% sensitive to Tetracycline.

Fourteen different isolates of C*itrobacter diversus* were tested against 6 different antibiotics. From this study 100% isolates were sensitive to Amikacin cotrimoxazole. Chloramphenicol, ofloxacin 71% were sensitive to tetracyctive, 50% sensitive to Nalidixic Acid whereas 28.6% were resistant to Nalidixic Acid and 21.4% were resistant do tetracycline.

Similarly all the 13 isolates of C*itrobacter freundii* were tested against 6 different antibiotics. Among which 100% were sensitive to chloramphenicol and ofloxacin 92.4% were sensitive to Amikacin 76.9% sensitive to contrimoxazole, 69.2% sensitive to Nalidixic Acid 15.4% were resistant to Cotrimoxazole, 7.7% were resistant to Nalidixic acid and Tetracycline.

Among the two isolates of *salmonella spp* 100% were sensitive to Amikacin, cotrimoxazole, chloramphenicol, ofloxacin whereas 50% were sensitive to Nalidixic Acid and Tetracycline only 50% were resistant tetracycline.

Five different isolates of *Salmonella* Typhi were tested against 6 different antibiotics. One of which 100% were sensitive to Amikacin, Cotrimoxazole, Chloramphenicol, 80% were sensitive to Nalidixic Acid, 60% sensitive to ofloxacin, 40% sensitive to Tetracyline.

Aarstrup et al. (2006) in his study isolated *Salmonella enterics* serotype schwarzengrund from food and food animals in Denmark and found to be resistance to Nalidixic Acid.

The study conducted by a bouzeed et al. (2001), Capital et al. (2003), Metzer et al. (1998), Winoker et al. (2000) also showed that tetracycline and Nalidixic acid was the least effective drug to treat *Salmonella spp.*

Various studies had been done in various parts of the worlds on antibiotic sensitivity test of organisms isolated from meat. In the study of NARC in the fiscal year 2042/2043, antibiotic sensitivity tests were carried out against different microorganisms *Staphylococcus aureus, E.Coli, Enterobacter, Edwardsiella, Citrobacter, Streptococcus, Diplococci*, and *Salmonella spp*. Isolated from the post mortem cases of Chicken. Among the antibiotics, Chloramphenicol was found to be the most sensitive against all the organisms followed by tetracycline, streptomycin, ampicillin and Gentamycin.

CHAPTER -VI
CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Among the isolates, *E.coli* was found to be the most predominant organisms. The present study indicates the sub standard quality raw meat sold at Kathmandu valley.

Among different localities, the meat sample collected from Kalimati, Chabahil, Satdobato, Tinkune and Putalisadak were found to be contaminated with highest number of bacteria in comparison to Kuleshwor and Swoyambhu showed the lowest bacterial count.

On antibiotic susceptibility test, it was observed that majority of the isolates were sensitive to common antibiotics such as Amikacin, Cotrimoxazole, Chloramphenicol, Ofloxacin, Nalidixic Acid. However few isolates were Nalidixic acid resistant.

6.2 Recommendations

Though the study was conducted with various limitations, here is some recommendation put forward on the basis of findings.

1. The meat shop should be allowed to establish only in clean and well kept surroundings away from the vegetable markets, garbage, public urinals and open drainages.
2. There should be the use of refrigerator or cold storage in order to check the microbial growth and use of net for protection from flies in meat shops.
3. Water used for molting, cleaning and washing should be of microbiologically safe quality and same water should not be used repeatedly.
4. Indiscriminate use of antibiotics to the slaughtering animals should be controlled.

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APPENDIX-I

LIST OF THE EQUIPMENTS USED DURING THE STUDY

1)	Microscope	Olympus (Japan)
2)	Incubator	NSW (India)
3)	Freeze	Toshiba (Japan)
4)	Autoclave	Life (India)
5)	Water bath	NSW (India)
6)	Oven	NSW (India)
7)	Weighing balance	A&D (Japan)
8)	Media	a) Enrichment– Selenite F-broth
		b) Isolation– PCA, NA, VRBA
		c) Differential- MA, XLD
		d) Biochemical media- Clark and Labs medium (MR/VP broth)
		Simmon's citrate medim
		SIM medium
9)	Chemical	H_2O_2
		Oxidase reagent
		Kovac's reagent

APPENDIX-II

PREPARATION OF CULTURE MEDIA

Different types of culture media such as enrichment media, selective media, and differential media were used. Composition and preparation of different types of culture media are given below.

1. Total Plate Count Agar (TPCA)

The total plate count agar is used for the enumeration of bacteria in foods and water.

Composition:

Ingredients	**Gms./Litre**
Tryptone	5.0
Yeast	2.5
Dextrose	1.0
Agar	15.0
Final pH (at 25^0C)	7.0±0.2

Direction for preparation:-

Suspend 23.5 grams of TPCA in 1000ml distilled water. Boil to dissolve the medium completely. Sterilize at 15 lbs pressure (121^0C) for 15 minutes.

2. **Nutrient Agar (NA)**

The nutrient agar is used for cultivation of pure culture for gram staining and various biochemical tests. It is also used for the preparation of blood agar.

Composition:

Ingredients	**Gms./Litre**
Peptone	5.0
Sodium Chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15.0
Final p^H (at 25^0C)	7.4±0.2

Direction for preparation:-

Suspend 28 grams in 1000ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121^0C for 15 minutes at 15 lbs pressure. Mix well before pouring.

3. Mac Conkey Agar (MA)

Mac Conkey Agar without salt and crystal violet and sodium taurocholate or bile salts is used for the detection and isolation of coliforms.

Composition:

Ingredients	**Gms./litre**
Peptone	20.0
Lactose	10.0
Sodium taurocholate	5.0
Agar	20.0
Neutral Red	0.04
Final pH (at 25^0C)	7.4±0.2

Direction for preparation:-

Suspend 55 gram of MA in 1000ml-distilled water, boil to dissolve completely. Sterilize by autoclaving at 121^0C for 15 minutes at 15 lbs pressure.

4. Violet Red Bile Afar (VRBA)

Violet Red Bile Agar is a selective medium for the detection and enumeration of coliform organisms. The VRBA is recommended for the direct plate count of coliform bacteria in water, milk, dairy and other food products. Coliform organisms produce dark red colonies, which are surrounded by red zone.

Composition:

Ingredients	Gms./litre
Yeast salt extract	3.0
Bile salt No.3	1.5
Peptone	7.0
Lactose	10.0
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.002
Agar	15.0
Final p^H (at 250C)	7.4±0.2

Direction for preparation:-

Suspend 41.5 gram of VRBA in 1000ml of distilled water. Heat to boiling to dissolve the medium completely. Cool to 45^0C and pour into dishes containing the vinculum. DO NOT AUTOCLAVE.

5. Mueller Hinton Agar (MHA)

This medium was originally formulated for the isolation of pathogenic *Neisseria spp*. (Mueller Hinton, 1941). Now days it is more commonly used in conjunction with high potency antiniotic disc for the determination of antibiotic sensitivity patterns by Kirby-Bauer technique (Bauer et al. , 1966).

Composition:

Ingredients	**Gms./litre**
Beef, infusion form	300.0
Caesin Acid hydrolysate	17.5
Starch	1.5
Afar	17.0
Final p^H (at 25^0C)	7.4±0.2

6. Nutrient Broth

Composition:

Ingredients	**Gms./litre**
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15.0
Final p^H (at 25^0C)	7.4±0.2

Direction for preparation:-

Suspend 28 grams of powder in 1000ml distilled water and then boil to dissolve completely. Then sterilize the medium by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

7. Selenite F enrichment broth

Selenite F enrichment broth is used as enrichment medium for members of *Salmonella* groups and few species of *Shigella* groups, when isolating these organisms from foods, dairy products etc.

Georgala and Boothroyd found that this medium is more selective for the isolation of *Salmonella* from foods when incubated at 43^0C instead of 37^0C. Proteus and paracolon bacteria are inhibited at 43^0C in this medium.

Composition:

Ingredients	**Gms./litre**
Part A	
Tryptone	5.0
Lactose	4.0
Sodium phosphate	10.0
Part B	
Sodium acid selenite	4.0
Final pH (at 25^0C)	7.4±0.2

Direction for preparation:-

Suspend 19 gram of part A and 4 gram of part B in 1000 ml distilled water. Warm to dissolve and mix well. Dispense and sterilize in a boiling water bath or in free flowing steam for 10 minutes. DO NOT AUTOCLAVE, excessive heat is detrimental.

APPENDIX-III

PREPARATION OF BIOCHEMICAL TEST MEDIA AND REAGENTS

1.Catalase Test:

Catalase test is done to test for the presence of enzyme catalase. The enzyme catalase splits hydrogen peroxide to water and oxygen. It is present in most cytochrome containing aerobic and facultative anaerobic bacteria; the main exception is *Streptococcus spp.*

Reagent: - 3% Hydrogen peroxide (H_2O_2)

Composition:-

Concentrated Hydrogen peroxide	3ml
Distilled Water	97ml

Procedure:-

Three milliliter of 3% hydrogen peroxide was taken in a test tube and a colony of bacteria to be tested was picked up from a nutrient agar plate with a help of a glass rod and inserted into the tube. The production of gas bubble indicates positive catalase test.

2. Oxidase test:

Oxidase test shows presence of enzyme oxidase, which catalyses the transport of electrons between electron donors in the bacteria and a redox dye tetramethyl-p-phenylene diamine dihydrochoride. The dye is reduced to deep, purple color.

Reagent:-

1% solution of tetramethyl-p-phenylene diamine dihydrochloride.

Preparation of dry filter paper:-

Since oxidase reagent is unstable it has to be freshly prepared for use. Strips of whatman no. 1 filter paper were soaked in a freshly prepared 1% solution of tetramethyl-p-phenylene diamine dihydrochloride. After draining for about 30 seconds the strips were freeze dried and stored in a dark tightly sealed bottle.

Note: - The reagent must be freshly made and the bacterial growth must be transferred to the test paper with a clean glass rod (or platinum loop) since traces of iron will catalyse the reaction and give a false positive result.

3. **Indole test**:

Indole test is done to determine the ability of organisms to split indole from tryptophan molecule with the help of various intracellular enzymes collectively known as "Tryptophanase". Indole is then tested for by a colorimetric reaction with p-dimethylaminobenzaldehyde.

Medium

Peptone (containing tryptophan)	20 gm
Sodium Chloride	5 gm
Distilled water	1000ml
P^H	7.4

Dispense and sterilize by autoclaving at 121^0C for 15 minutes.

Kovac's Reagent

Amyl or Isoamyl alcohol	150ml
p-dimethyl-aminobenzaldehyde	10gm
Conc. Hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator, shake gently before use.

Procedure:-

Inoculate the medium and incubate for 48 hours at 37^0C. After incubation add about 0.5ml Kovac's reagent and shake gently. A red color in the alcohol layer (a red ring) indicates a positive reaction.

4. Methyl Red Test:

The methyl red test is employed to detect the production of stable acid end products from glucose fermentation and to overcome the buffering capacity of the system. The p^H decreases below 4.5, which can be shown by a change in the color of the methyl red indicator,

Medium (Glucose phosphate, peptone water)

Buffered peptone	7.0gm
Dextrose	5.0gm
Potassium phosphate	5.0gm
Distilled water	1000gm
Final p^H (at 25^0C)	7.4±0.2

Dissolve 17 gram in 1000ml distilled water. Distribute in test tubes in 10ml amount and sterilize in autoclave at 121^0C for 15 minutes.

Methyl Red solution

Methyl red indicator	0.1gm
Ethanol	300ml
Distilled water	200ml

Procedure:-

Inoculate freshly young bacterial culture onto the liquid medium and incubate at 37^0C for 48 hours. Add about 5 drops methyl red indicator immediately. Positive test is shown by red color, while negative test by yellow color.

5. **Voges-Proskauer Test:**

VP test is done to demonstrate the ability of some organism to produce a neutral end products acetyl methyl carbinol (acetoin) or its end product 2,3-butylene glycol from glucose fermentation. The substances can be tested for by a colorimetric reaction between diacetyl ($CH_3CO.COCH_3$ formed during the test by oxidation of acetyl methyl carbinol or 2,3 butylene glycol) and a guanido group under alkaline condition.

Medium:-

Glucose phosphate peptone water is used as for the methyl red test. Detection of VP test by Barritt's method.

Procedure:-

To 5 ml of 48 hours old culture, 1ml 40% KOH and 3ml and α-naphthol solutions in absolute alcohol were added. A positive was indicated by cosin pink color in 2-5 minutes.

6. Citrate Utilization Test:

Citrate Utilization test is done for the ability of certain organism to utilize citrate as the sole source of carbon and energy source for the growth. The ability to use citrate can be used to differentiate among the members of the Enterobacteriaceae.

Medium	**Gms./liture**
Magnesium sulphate	0.2
Sodium Citrate	5.0
Ammonium dihydrogen phosphate	1.5
Potassium dihydrogen phosphate	1.0
Sodium Chloride	5.0
Agar	20.0

Bromothymol blue (0.2%) 40ml

Dispense autoclave at 121^0C for 15 minutes and allow setting up slopes.

Procedure:-

Since the reaction required oxygen, the organism was inoculated to the surface of the agar slant of the medium. A very light inoculum was picked up with straight wire (to prevent false positive reaction) and streaked over slant, and incubated for 24 hours at 37^0C with LOOSE CAP.

Growth of organism on the slant with reversion of the color indicator from green to blue was evidence of positive test, indicating that the organism was able to grow and produce acetate and other carbohydrates and products.

Note:- There may be a rare citrate positive organism that can utilize the substance without producing enough alkaline reaction to change the p^H indicator. Luxuriant growth on the slant without a blue color may indicate the positive test, but the test should be repeated with animal inoculums.

7. Urease Test:

Urease test is done to split urea, forming two molecules of ammonia, by the action of enzyme urease. Bacteria particular those growing naturally in an environment exposed to urine, may decompose urea by means of the enzyme urease.

$NH_2.CO.NH_2.H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$

The occurrence of this enzyme can be tested for the growing the organisms in the presence of urea and testing for alkali (NH_3) production by means of suitable pH indicator. (Phenol red)

Medium	**Gms./lit**
Peptone	1.0
Dextrose	1.0
Sodium chloride	5.0
Disodium hydrogen phosphate	1.2
Potassium dihydrogen Phosphate	0.8
Phenol red	0.012
Agar	15.0
Final p^H	7.1±0.2

24 grams was suspended in 950 ml distilled waster and sterilized by autoclaving at 115^0C for 20 minutes. After cooling to about 55^0C ml of 40% urea was added and mixed well. Then 5 ml was dispesed in test tube and set at slope position to make urea agar slant.

8. Tiple Sugar Iron Agar (TSI) Slant

Medium	**Gms./litre**
Peptone	10.0
Tryptone	10.0
Yeast extract	3.0
Beef extract	3.0
Lactose	10.0
Saccharose	10.0
Dextrose	1.0
Ferrous sulphate	0.2
Sodium Chloride (NaCl)	5.0
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0
p^H (at 25^0C)	7.4±0.2

Procedure:-

65 gram was dissolved in 1000 ml distilled and boiled to dissolve the medium completely, which was then sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. The medium was allowed to set in slop form with a butt about 1 inch of long.

9. Hugh and Leifson's Medium

Medium	**Gms./litre**
Tryptone	2.0
Sodium Chloride	5.0
Dipotassium phosphate	0.3
Bromothymol blue	0.08
Agar	2.0
Final p^H (at 25^0C)	6.8±0.2

9.4 grams were suspended in 1000 ml distilled water. The medium was boiled to dissolve the medium completely. Dispensed in 100 ml amounts and sterilized at 15Ibs pressure (121^0c) for 15 minutes. To 100ml of sterile medium, aseptically sterile carbohydrate (dextrose) solution was added to a final concentration of 1%. Mix each flask thoroughly and aseptically dispense in 5 ml amounts into sterile tubes.

BIOCHEMICAL TEST USED FOR IDENTIFICATION OF BACTERIA:

After obtaining pure culture, colony was passed into nutrient broth and incubated at 37^0C for 4 hours, then inoculated into different biochemical media for different biochemical test as given below.

For each set of biochemical test, both positive and negative control stains were inoculated and one uninoculated media was also incubated and examined along with the test.

The following tests were done:-

(i) Catalase Test (Mc Cartney, 1989):

This test demonstrates the presence of Catalase, an enzyme, that catalyses the release of Oxygen from Hydrogen Peroxide. On a clean dry slide one drop of 3% Hydrogen Peroxide (H_2O_2) was taken and with the help of a sterile glass rod, a colony was picked and smeared with H_2O_2 and the bubbling of the gas was noted down.

(ii) Oxidase Test (Mc Cartney, 1989):

This test depends on the presence in bacteria of certain oxidases that will catalyze the transport of eelectrons between electron donors in the bacteria and the redox dye-tetramethyl paraphenyl diamine dihydrochloride. The oxidase test papers were moistened with distilled were a colony from the fresh culture was picked up with a sterile glass rod and rubbed on the paper. Development of violet purple color within 10 sec is an indicator of positive test.

(**iii) Voges Proskauer Test (VP-Test) (Mc Cartney, 1989):**

This is the test to determine the ability of the organisms to ferment carbohydrate with the formation of Acetyl Methyl Carbinol (Acetone). The MR-VP broth (Glucose phosphate Peptone Water Medium) was dispensed in 5 ml tubes and sterilized. The test organisms were inoculated and incubated for 48 hours at 37^0C. After incubation Barritt's Reagent (0.6 ml of 5% α-naphthol in absolute alcohol and 0.2 ml of 40% KOH) were added to each tube and shaken well and kept for 15 minutes. Reddening of the medium within 15 minutes indicates the positive test (But in some cases it takes long time for the development of red color).

(iv) Indole Production Test (Mc Cartney, 1989):

This test demonstrates the ability of certain bacteraia to decompose the Amino acid-Tryptophan to Indole that accumulates in the medium. Test tubes with SIM medium (sulphide-indole-motility) were stabbed with fresh culture from Nutrient Broth and then incubated at 37^0C for 48 hours. After incubation 2-3 drops of Kovac's reagent was added and the appearance of red color in the alcohol layer indicates a positive reaction.

APPENDIX-IV

PROTOCOL

1. Type of sample: Sample No.:

   Sampling time: Date:

2. Sample Site:

3. Hygiene of handler (Butcher)

   Cloth: Hands:

   Other:

4. Environmental condition of the sampling site:

   i. Utensils:
   a. b. c.
   ii. Water used
   a. b. c.

5. Physical appearance of sample:

   i. Colour
   a. b. c.
   ii. Odours:
   a. b. c.
   iii. Others:
   a. b. c.

Processing of sample

S.N.	Media	Dilution	No. of colony
1.	TPCA/NA		
2.	TPC/NA		
3.	VRBA		
4.	VRBA		

Enrichment of samples

S.N.	Media used	Incubation period	Observation	Result
1.	Peptone			
2.	Tetrathionate broth			
3.	Sellenite F broth			

Sub culture of S-F/Tetrathionate broth on XLD/SS agar

Colony character

S.N.	Size	Colour	Margin	Elevation	Consistency	Opacity	Configuration
1.							
2.							
3.							
4.							
5.							
6.							

Microscopic study (observation)

S.N.	Colour	Shape	Size	Gram Stain
1.				
2.				
3.				
4.				

Biochemical Character

S.N.	Catalase	Oxidase	TSI	SIM	MR	VP	Citrate	Urase	Result
1.									
2.									
3.									
4.									

Antibiotic Sensitivity Test

S.N.	Antibiotic	Zone of Inhibition	Result
1.			
2.			
3.			
4.			
5.			

Serology of Salmanella sps.

S.N.	Slide Agglutination test				Result
	Negative	Test organism	Positive control	Observation	

APPENDIX-V

ZONE SIZE INTERPREATIVE CHART

Antimicrobial Agents used	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Susceptible (mm or more)
Amoxycillin	Ak	30mcg	14	15-16	17
Cotrimoxazole	Co	25mcg	10	11-15	16
Chloramphenicol	C	30mcg	12	13-17	18
Oflozacin	Of	5mcg	12	13-15	16
Nalidixic Acid	Na	30mcg	13	14-18	19
Tetracycline	T	30mcg	15	15-18	19

(Sources: Product information guide, Hi-Media Laboratories Pvt. Limited, Bombay, India)