BACTERIOGICAL QUALITY OF PORK MEAT MARKETED IN DHARAN SUB-METROPOLITAN CITY

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Bacteriological Quality of Pork Meat Marketed In Dharan sub-metropolitan city

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Approval Letter

This *dissertation* entitled *Bacteriological Quality of Pork Meat Marketed in Dharan sub- metropolitan city* presented by Bimal Pariyar has been accepted as the partial fulfillment of the requirement for the B.Tech. Degree in Food Technology

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Abstract

The present work was undertaken to assess the bacterial quality of pork meat marketed in Dharan sub-metropolitan city. Pork meat, swabs of knives, swabs of chopping board and swabs of hands of butchers were examined for microbiological parameters (TPC, total Coliforms, *E. coli, S. aureus, Salmonella* and *Shigella*). A survey with the help of questionnaire was done to assess the sanitary condition and personal hygiene of meat shops and butchers.

Average value for TPC of meat sample was found to 181×10^5cfu/g. The average coliform, *E. coli* and *Staphylococcus aureus* counts were 918×10^3, 862 and 473×10^1 cfu/g respectively. Except three samples, all samples were found to be infected with *Salmonella* where as six out of seven samples were found to be contaminated with *Shigella*. The average value for total plate count of Chopping board, knives and palms of butchers were found to be 287×10^2, 494×10^1 and 274×10^2 cfu/cm^2 respectively. The average coliform, *E. coli* and *Staphylococcus aureus* counts of chopping boards were found to be 133×10^1, 50 and 14×10^1 cfu/cm^2. The average Coliform, *E. coli* and *Staphylococcus aureus* counts of knives were found to be 106×10^1, 54 and 80 cfu/cm^2. The average coliform, *E. coli* and *Staphylococcus aureus* counts of the palms of butchers were found to be 176×10^1, 198 and 271 cfu/cm^2. Five swabs of chopping board, six swabs of knives and three swabs of hands were *Salmonella* free. Out of seven swab samples four samples of chopping board, one sample of knives and two samples of hands *Shigella* was detected from .The field survey of 35 meat shops showed that the hygienic condition of meat sold in Dharan metropolitan city was found unsatisfactory.

Keywords: Pork meat, Hygiene, TPC, Coliforms, *S. aureus, Salmonella, Shigella*

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List of abbreviations

Abbreviations	Full form
AOAC	Association of Official Analytical chemist
DFD	Dark, Firm and Dry
EU	European Union
FAO	Food and Agriculture Organization
HACCP	Hazard Analysis and Critical Control Point
LSD	Least Significant Difference
PSE	Pale, Soft, and Exudative
WHO	World Health Organization

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Part I

Introduction

1.1 General Introduction

Hygiene is a set of practices performed for the preservation of health. According to the World Health Organization (WHO), "Hygiene refers to conditions and practices that help to maintain health and prevent the spread of diseases. Meat hygiene is the creation of conditions and implementation of measures necessary to ensure the safety and suitability of meat at all stages of the meat production chain (Chambers and Grandin, 2001).

Meat processing hygiene is part of Quality Management (QM) of meat plants and refers to the hygienic measures to be taken during the various processing steps in the manufacture of meat products. Regulatory authorities usually provide the compulsory national framework for food/meat hygiene programs through laws and regulations and monitor the implementation of such laws. At the meat industry level, it is the primary responsibility of individual enterprises to develop and apply efficient meat hygiene programs specifically adapted to their relevant range of production (Narasimha Rao and Heinz, 1991)

A well-planned, well-executed and controlled cleaning and sanitation program for rooms, machines and equipment is very important to achieve a hygienic standard. Cleaning and sanitation alone, however, will not assure a hygienic standard in production where process hygiene as well as personal hygiene are important factors (Wilson et al., 1981).

The microbiology of carcass meat is highly dependent on the conditions under which animals are reared, slaughtered and processed. The extent to which contamination occurs and the composition of the flora that results reflects the standard of hygiene in the slaughterhouse (Wilson et al., 1981) . The hide or skin of the particular animal and others being dressed in its close proximity is probably the major source of saprophytic species. In addition to skin, the gastro-intestinal and respiratory tracts, urine and milk are other important animal sources of infection. Generally *Escherichia coli* comprise a greater proportion of the total aerobic flora of the intestine than of the hide or fleece (Brown, 1982).

After slaughter and evisceration animal meat retains the general microbial characteristics that it had prior to slaughter. The surface of the animal is contaminated with soil, air and water borne organisms. Extremely high numbers of microorganisms are found in the animal's intestinal content, and it is expected that some of these will find their way to the surface of the carcass during the dressing operations (Price and Schweigert, 1971).

The factors influencing the growth of microorganism in meat are the physical properties of the meat, chemical properties of the meat, availability of oxygen and temperature (Prasai, 2000).

Foods of animal origin are the primary source of many bacteria responsible for food borne infections and intoxications. Organisms found in the live animal can be carried through raw meats after slaughter, may persist through further processing and ultimately may appear in the final retail product if insufficient attention is paid to hygiene and temperature control (Karki, 1995).

The nature and level of microbial contamination in meat have important consequence in relation to public health, storage life and the type of spoilage of meat (Gracey and Collins, 1994).

Raw meat quality is often judged by the size of its microbial population able to grow at 30-37°C. However, this count is most appropriately used to monitor hygiene not quality (Bobbitt, 2002).

Despite a large number of control measures along the meat chain, meat contaminations by *Salmonella* and pathogenic *E. coli* remain a serious public health problem in humans. In the pre-slaughter stages of the meat chain, difficulties in identifying asymptomatic shedders constitute the main obstacle to the control of the infection spread in live animals (Wilson *et al.*, 1981). Further studies are therefore needed to identify cost-effective techniques and approaches to diagnose asymptomatic carriers in cattle herd before animal transportation to abattoirs. During the slaughtering process, the skinning and evisceration operations appears to be the most critical stages for carcass contamination (Bobbitt, 2002). Thus Good Manufacturing Practices in accordance with HACCP principles must be strictly applied in commercial slaughterhouses to reduce the risk of carcass contamination at those specific stages. The decontamination of carcasses has also shown a potential in reducing pathogen numbers on carcasses prior to chilling, even if its utilization in some countries is

still limited by a number of factors including the cost of installations, the commercial quality of treated carcasses as well as the risk of relying only on the carcass decontamination step and reduce efforts devoted to Good Hygiene and Manufacturing Practices in previous slaughtering stages (Price and Schweigert, 1971). Along post-slaughter stages of the chain, handling, time and temperature are the main factors influencing the microbial contamination of meat. Therefore application of appropriate GMP and GHP by meat processing plants is of great importance to prevent cross-contaminations during cutting/boning, processing, transportation and retail of meat products (Niyonzima *et al.*, 2015).

The possible sources of contaminating bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipment used for each operation that is performed until the final product is eaten, the clothing and hands of personnel and the physical facilities themselves are all implicated (Rombout and Nout, 1994). Retail cut could also result in greater microbial load because of the large amount of exposed surface area, more readily available water, nutrient and greater oxygen penetration available hence retail cuts displayed are conducive for microbial growth and proliferation which leads to spoilage of the meat (Adzitey *et al.*, 2011).

Dharan Sub-metropolitan (192.61km^2 area) situated on the foot hills of Mahabharat range is second largest city in eastern region of Nepal (Dharan sub-metropolitan city office). The consumption of pork meat and meat product is increasing day by day with increase of population. According to Pig Entrepreneur Association Nepal, 2016 people ,here, consume 3850 kg of pork meat (approx. 55 pigs) per day, but hygienic standards of meat and meat shops are not satisfactory.

1.2 Statement of the problem

Our health mostly depends on what we consume, especially our food which determines our health. Evaluation of quality before consuming is a must for any food, and this is more so for commodities like meat, meat and egg: they are complete foods in themselves and support not only our life but also that of pathogenic microorganisms. Microbiological quality is important from public health point of view. Both consumers and sellers of Dharan metropolitan city are not very sensitive towards meat quality. Apparently the

hygienic condition of meat sold in Dharan sub-metropolitan city market is very poor because there is no facility of properly managed slaughter house, no practice of postmortem and ante-mortem inspection of meat. Use of unsterilized equipment, Lack of fencing and maintaining clean environment around the shop, personal hygiene of shop personnel are the main evil in maintaining hygienic standard of meat. Consumption of unhygienic meat causes food poisoning due to the entrance of pathogenic and food poisoning bacteria. Although food poisoning incidences due to consumption of poor quality meat has not been recorded systematically to date, this does not necessarily imply that meat sold in Dharan sub-metropolitan city is safe. In fact, no such systematic studies have been made on pork meat market of Dharan sub-metropolitan city so far, let alone the recorded incidences of zoonotic diseases.

1.3 Objectives

1.3.1 General objective

The general objective of the present work is to study the general condition of meat market and hygienic quality of pork meat marketed in Dharan sub- metropolitan city.

1.3.2 Specific objectives

The specific objectives of this work:

The specific objectives are as follows:

1. To analyze microbial quality of pork meat.
2. To enumerate the pathogenic flora of pig meat, e.g., *Salmonella*, *Shigella*, total *coliforms*, etc
3. To determine possible contaminating sources.
4. To enumerate the microbial load on possible contaminating sources.

1.4 Significance of the study

Study of hygienic quality of pork meat and improvement in microbial standard will benefit both consumer and seller of pork meat. This study may also encourage local people and local Government to take steps towards the healthy and hygienic meat consumption. People may suffer from different zoonoses, food poisoning and health hazards by the consumption of unhygienic meat. Meat seller may suffer economic loss due the

contamination of meat. This study is helpful to aware the people about the health and economic loss from unhygienic meat processing and handling practices. It will reflect the actual condition of pork meat in terms hygienic quality and milestone for the concern authority to run the programs to protect health of consumer.

1.5 Limitations of the work

- Survey was limited to some part of Dharan metropolitan city.
- The study is limited to the investigation of some common pathogenic bacteria like *Salmonella* and hygiene indicators like total *coliform.*

Part II

2. Literature review

2.1 Meat

Meat is a very nutritious food. It is almost fully digestible. The nutritive value of meat is attributed to its abundant high quality protein, essential fatty acids, some important minerals, and B-complex group of vitamins (Sharma and Mendiratta, 1999). When cooked lean meat supplies nutrients which contribute significantly to the dietary balance of meal. As little as 100 g of lean meat provides half of the protein needed for a day and the amino acid content of this protein is such that it compensates for deficiencies common in the protein of vegetables and cereal products. Such a small quantity of lean meat also contains only about 200 calories of energy; hence the use of meat is important in the modern dietary practice of using variety of foods to provide ample quantities of each nutrient without an excessive caloric intake (Rice, 1971).

Meat contains 20-22% protein, which is considerably higher than most of vegetables and animal products. It is reach in essential amino acids (Subba, 2002). Meat and meat products are rich source of proteins, peptides and amino acids for bacteria as well as for human. Thus it is not surprising that bacteria capable of metabolizing these materials are encountered commonly on and in meat products (Lechowich, 1997).

Because of essential nutrient it contains, meat eaten in moderate amounts can be considered a valuable component of complete diet and should present little risk to health. As it is also ideal culture medium for growth of various microorganisms because of its high moisture content, rich nitrogenous foods, plentiful supply with minerals, accessory growth factors and favorable pH (Frazier and Westhoff, 1997).

2.1.1 Quality of meat

There is not a single definition which can fully describe the "quality of meat". Health and ethical aspects may be as important as technological and sensory characteristics of the meat. Together they form what we call ‘meat quality’. To buy a piece of meat, factors like tenderness, juiciness, color and taste referred generally as eating quality will become more important. When it comes to processing factors like pH and water holding capacity will be of paramount importance (Adhikari *et al.*, 2012).

Meat quality is normally defined by the compositional quality (lean to fat ratio) and the palatability factors such as visual appearance, smell, firmness, juiciness, tenderness, and flavor. The nutritional quality of meat is objective yet "eating" quality as perceived by the consumer, is highly subjective (Subba, 2010b).

The factors affecting meat quality are lairage condition of animal, pre-slaughter handling, slaughtering practices, post slaughter handling, transportation, micro flora, genetics, nutrition, animal handling and slaughter weight. Among all these factors, one of the most important factors is slaughtering practices. Slaughtering practices affect the color flavor, microbiology, water holding capacity, cooking loss and pH of meat (Acharya, 2010).

Hygienic standard of processing, handling, and marketing of meat is responsible for growth of micro-organism in meat. Contact of meat with skin, care during evisceration process, sterilization of equipment personal hygiene of meat personnel and sanitary condition of shop etc., determines extent of contamination of meat by micro-organism like *E. coli., Staphylococcus areus, Salmonella, Shigella etc* (Subba, 2010b).

2.1.2 Factors affecting hygienic quality of meat

There are several factors affecting hygienic quality of meat. Adequate personal hygiene assures the overall cleaning process. Deterioration of the cleaning standard may occur if microorganisms are transmitted to well-cleaned surfaces from unwashed hands before processing starts. Neither process hygiene, personal hygiene nor cleaning and sanitation alone can assure a sufficient hygienic standard but together, if carried out in an optimal manner, they will guarantee a complete hygienic standard (Beach *et al.*, 2002; Dave and Ghaly, 2011).

2.1.2.1 Process hygiene

It is impossible to give an adequate definition of process hygiene because the critical points will vary, depending on processing, processing buildings (site, size, buildings), equipment available, permanent or non-permanent personnel (working routines, training), climatic conditions, sanitary facilities, water and energy supplies, liquid and solid waste disposal (Bolton *et al.*, 2002).

2.1.2.1.1 Site of buildings for slaughtering and processing

The slaughterhouse should be situated away from residential areas. Access for animals - either by road, rail and/or stock route must be assured. The slaughterhouse should be located in areas where flooding is impossible. An abundant supply of potable water as well as adequate facilities for treatment and disposal is important. The land acquired for the proposed slaughterhouse should be sufficient to permit future expansion as over-crowding of facilities may give sanitation problems. Where the "slaughterhouse" is more or less an open slaughter place, trees may provide some shade or even be used as a part of the structure. If the slaughterhouse consists of regular buildings the ground should be free of shubbery or vegetation in close proximity to the structure (FAO, 1978; Mann, 1984).

2.1.2.1.2 Size

There should be a reasonable relationship between the size of slaughter facilities and the number of animals to be killed. Sufficient space for lairage and tripe and hide treatment is required. The space required for lairage will often depend on local and even climatic conditions. In specific areas it will only be possible to transport the animals in the dry season while slaughtering may only be carried out in the rainy season because of water requirements. Sufficient space is required to dig pits for condemned animals, compost stacks, lavatories etc., and for disposal of liquid and solid waste (FSAI, 2009).

2.1.2.1.3 Buildings / facilities

Buildings / facilities should be so constructed that clean and unclean processes and products do not mix. The floor must be hard, smooth and impervious, sloping sufficiently towards a drain thus allowing cleaning with water. Walls, if any, may be made of local construction materials. In certain dry areas walls are not necessary. Materials, which can be cleaned by water, are recommended, e.g. stone, lava blocks, bricks or concrete. Roofs, if any, may be constructed of materials available (tiles, corrugated iron, asbestos or Al) (FAO, 1978).

2.1.2.2 Equipment

The main principle for equipment such as tables, hooks and machines, etc. should be that it is easy to dismantle or remove to facilitate cleaning and that it should be made of non-corrosive materials. Essential for the hygienic handling of carcasses and meat is equipment

for hoisting the carcasses, when slaughtered. Hoists, when possible, should be preferred to working tables. Procedures assuring a periodical or continuous cleaning of hoists are recommended. Cleaning and disinfection will often be complicated or impossible because of the complex construction of machines and when choosing and buying machines, hygienic production and possibilities for cleaning and disinfection must be considered (FAO, 1978).

2.1.2.3 Permanent or non-permanent personnel

Many large slaughterhouses have permanent personnel performing all work in the slaughterhouses. Organization will depend on the type of production. Where personnel is permanent, a few lessons regarding process hygiene, personal hygiene, and cleaning and disinfection may be given. Ideally, personnel should be organized in a way that part of the staff is occupied with cleaning and disinfection. This group of personnel must be educated and trained especially in cleaning and disinfection procedures as well as general hygiene. Where the slaughterhouse/slaughter facilities cover the need for slaughtering in big areas in developing countries, the slaughterhouse is often owned by municipalities and organized with a manager and no permanent staff. Slaughtering is done by local butchers and a team employed by these butchers and thus it will be difficult to give them sufficient education and training in hygiene. It is therefore recommended that the manager of the slaughterhouse/slaughter facilities employ a team which is responsible for maintenance of a hygienic standard. This team should do some clearing and cleaning during slaughtering hours or instruct the butchers and workers to do this during and after slaughtering. This team will be responsible for cleaning and disinfection at the end of the working day and in maintaining the hygienic standard (FAO, 1978; FSAI, 2009).

2.1.2.4 Sanitary facilities

Water points, hoses, sterilizers for hand tools etc. and cleaning equipment must be provided in sufficient numbers. Where possible sterilizers should be supplied with hot water instead of chemical disinfectants. Sanitary facilities must also include a sufficient number of toilets/latrines and arrangements for hand-washing or even possibilities for bathing (showering). These facilities must be kept clean and well maintained. To avoid back-flow from toilets in case of flooding the toilet outlets must be separated from

common waste water outlets. Areas/rooms for resting and eating may be required assuring that food for the personnel and the carcasses/meat cannot be mixed (Mann, 1984).

2.1.2.5 Water and energy supplies

If sufficient water of drinking quality is available, it will be possible to plan processing and cleaning procedures in a way which assures hygienic products. The water supply may be from the premises own well or from the community supply. Working routines should be planned to economize the consumption of water because of waste water disposal. Energy supplies will be necessary if the slaughterhouse is more or less automatic. Energy supplies will also be necessary for automatic cleaning and could be provided through windmills, biogas production, fuel and electricity and water could also be heated by solar energy. If water and energy supplies are sufficient it will be the responsibility of the management of the slaughterhouse to see that these supplies are used efficiently and that sufficient water and energy are used for hygienic purposes (Mann, 1984).

2.1.2.6 Processing

The elements of hygiene will differ depending on the type of processing. There will be significant differences between the hygiene standard required in a plant manufacturing meat products, which are sold as sliced, prepackaged meat products, and the hygienic standard required in a place where the animals are slaughtered. The main hygiene principle in processing is that clean and unclean operations are efficiently separated. This requires a well planned plant layout, where the purpose of any structure should be to protect the products against unintended contamination (Arthur *et al.*, 2007).

2.1.2.6.1 Stunning, slaughtering and bleeding

These processes must, if possible, be separated from the operations which follow. If the blood is not intended for use it should be drained away into a separate pit and should not be allowed to drain into the waste water. The animals should be hoisted to facilitate bleeding and decrease the risk of contamination of the carcasses. This area should be constructed with a slope towards drains (FAO, 1978).

2.1.2.6.2 Scalding, skinning, dehairing or plucking

The process varies according to animal (poultry, pigs and cattle). Skinning or dehairing may be carried out in a separate room/area or in the slaughtering place. However, it should be carried out separately from the evisceration process (Subba, 1996). The same principle applies to plucking of poultry. The deskinned/dehaired carcass must never enter the clean area, but as soon as possible after skinning, dehairing, or plucking, it must be hygienically transferred to the clean area (evisceration room/or area). It is important to handle the carcass carefully to minimize contamination. To secure and improve cleanliness and efficiency hoists and overhead rails are required for the skinning/dehairing process. If hoists and over head rails are not available, the carcasses should be kept above floor level by means of cradles. Procedures assuring cleaning of hoists, over head rails and cradles should be established. Special rooms/areas should be available for treatment of hides (Subba, 1996).

2.1.2.6.3 Evisceration

During the evisceration process care should be taken to minimize contamination. Special care must be taken to avoid damaging the intestines. Edible organs must be handled in a hygienic way (stored/ removed in separate containers etc.). Waste must be removed rapidly from the floor in the evisceration room/area. A sufficient number of sterilizers for hand tools, knives, etc. must also be available in the evisceration area (Warriss, 2010).

2.1.2.6.4 Cutting /deboning

If cutting and/or deboning is carried out care must be taken to minimize contamination of the meat. The carcasses must be cut, preferably hanging or on surfaces (tables, cutting planks, chopping blocks), which are regularly cleaned. A sufficient amount of sterilizers must be available for cleaning of hand tools, knives, etc. The meat must be removed and/or stored in clean containers, which solely are used for meat. Disposable containers will assure hygienic transport and storage, but will be costly (Warriss, 2010).

2.1.2.7 Environmental hygiene

Environmental hygiene and its implementation will depend on the area where the slaughterhouse/meat plant is situated. The precautions to be taken will be different if the site is in a town or in the country. The main principles of environmental hygiene will

consist of proper fencing (public, dogs, etc.), pest control (rodents, insects), liquid and solid waste disposal (Dave and Ghaly, 2011).

2.1.2.8 Personal hygiene

Personal hygiene will usually be the main element in the term "hygiene"; the reason being obvious. Bacteria causing diseases or spoilage may be carried and transmitted to surfaces and food by workers handling the food products (Bolton *et al.*, 2002). Careful and frequent hand-washing will do much to reduce contamination. Hands washing before work starts, after using the toilets, after touching dirty objects/materials and after smoking and eating must be done properly. The clothing of slaughterhouse workers must be clean. The purpose is not to protect the worker against contamination but to protect the meat/food against contamination (Dave and Ghaly, 2011). Human hair and beards are normally heavily contaminated with bacteria and to prevent contamination of food, a hair or beard covering in the process area is a necessary part of the working clothes. If the use of gloves is indicated they must be kept in the same good hygienic conditions as hands, otherwise it is better to avoid their use. Good health is important for workers in the meat industry. Ill persons will often be carriers of more microorganisms (pathogenic microorganisms) than is usually the case. These microorganisms may then be transmitted to the meat/food with the risk of causing disease to the consumers (Bolton *et al.*, 2002).

2.1.2.9 Microbiological quality

Contamination of sterile animal muscle used as food is a direct consequence of slaughtering and dressing of animal carcasses. Wide ranges of microorganisms from different sources are introduced onto moist muscle surfaces that are rich in nutrients. It is argued that only a small portion (10%) of these microorganisms is capable of survival and proliferation during storage, distribution, and retail sales of meats. Additionally, an even a smaller portion will eventually predominate and cause spoilage (Cohen *et al.*, 2007).

Meat can be contaminated by two ways viz. intrinsic contamination and extrinsic contamination. The word intrinsic describes the microbial flora occurring in deep tissues and extrinsic describes the contamination received during dressing and handling. Meat demands strict hygiene during slaughter and further processing. It is an ideal culture medium for many microorganisms because it is high in moisture, rich in nitrogenous foods of various degree of complexity and plentifully supplied with minerals and accessory

growth factors. Also it usually has some fermentable carbohydrate and is at a favorable pH for most microorganisms (Bacus and Brown, 1981).

Organisms will physically and chemically alter the substrate on which they grow, producing unwanted odors, tastes and colors. If mold contaminants are present, then visible mold colonies may develop. Among some of these contaminants, frequently will be bacteria capable of causing disease or producing toxins dangerous to the human consumer. Ever since food poisoning statistics have been produced, meat and poultry dishes have been prominent as vehicles of illness (Wilson *et al.*, 1981).

The characteristic microbial populations developing in meat products are the result of the effects of the prevailing environmental conditions on growth of the types of microbes initially present in the raw materials or introduced by cross contamination or processing (Ford and Park, 1980).

2.2.1 Sources of contamination

Bacterial contamination of carcasses may occur at virtually every stage of slaughtering and processing. Processing hygiene, however, aims at holding the initial bacterial numbers on a level as low as possible, since this affects shelf-life as the occurrence of pathogenic bacteria (Upmann *et al.*, 1878).

Microorganisms have been found in the lymph nodes, bone marrow and even flesh of healthy animals. Staphylococci, Streptococci, *Clostridium* and *Salmonella* have been isolated from the lymph nodes of red meat animals, *E. coli* from intestine and hide, Clostridia spp. from livers and pancreas of apparently healthy animals. Ante-mortem infection may be increased by starvation, fatigue and shock (Nottingham, 1982).

The exterior of the animal harbors large numbers of many kinds of microorganism from soil, water and manure, as well as its natural surface flora. Molds, mainly *Cladosporium*, *Sporotrichum*, *Mucor*, etc; yeasts, mostly *Asporogenous* and bacteria, mostly *Micrococcus*, *Bacillus*, *Clostridium*, *Escherichia*, *Salmonella*, etc may reach the surface of meats and grow there (Subba, 2010b).

The microbial spoilage of meat is influenced by the original bacterial content present in it and by secondary contamination during processing. Secondary contamination is mainly due to using contaminated equipments (cutting tools, chopping blocks, containers etc), the

surrounding air and water and carrying agency. Man, as a carrier of different organisms, is the most important factor in the area of secondary contamination (Heinzal *et al.*, 1987).

Knives, cloths, air and hands and clothing of workers serve as intermediate sources of contamination. During handling of meat thereafter, contamination can come from boards, boxes, or other containers, form contaminated meat, from air, and from personnel (Frazier and Westhoff, 1997).

The essential problem in many developing countries is the failure to provide for hoists or hooks, hardware which permits the dressing of carcasses to take place off the floor. The contamination resulting from floor dressing of carcasses is considerable, especially where the removal of hides and the cleaning of stomachs are carried out in the same location as the dressing of the carcass itself (Mann, 1984).

Personal hygiene and particularly keeping the hands clean are important in relation to the spread of *Salmonella* of pathogenic varieties (Heinzal *et al.*, 1987). Holding animals in vehicles or lairages without adequate litter and/or drainage frequently results in fecal soiling of the skin. Animal for slaughter are often very dirty, their legs covered with manure. In these cases, the knife will have to cut through manure and fecal residues, resulting in a great possibility for meat contamination (Chambers and Grandin, 2001).

Coliform bacteria, Gram negative mesophiles and psychrophiles and enterococci are often used as indicators of good plant hygiene (Brown and Baird, 1982).

2.2.2 Microorganisms of public health concern

2.2.2.1 Aerobic mesophillic bacteria

The total plate count (TPC) expressed as organism/g on fresh meat or a meat product sets a limit to its shelf life. Meat will spoil with TPC at 10^6/g because of off odours. Slime and discoloration appear at 10^8/g (Anon., 2003).

The most commonly used hygiene indicator to investigate the persistence of specific spoilage or pathogenic organisms is the total aerobic mesophillic count(30^oc) (Brown, 1982).

Almost all food poisoning bacteria and most spoilage causing bacteria are mesophiles. A high TPC resulting from severe contamination during slaughter or processing will

shorten the shelf life even in ideal conditions. It also indicates poor hygiene so that contamination with food poisoning bacteria is likely (Narasimha Rao and Heinz, 1991).

According to Inspected German Quality meat, maximum value for the TPC for fresh meat on cutting and packaging unit is 5×10^6 cm^2 or g and the value is same for EU microbiological standards of cut meat for retail sale and further processing also. Danish Quality Assurance Warranty specifies freshly slaughtered meat must contain TPC on an amount less or equal to 10^4/sq. cm or g (Anon., 2003).

2.2.2.2 Coliforms

Members of total coliforms and fecal coliforms groups are referred to as indicator organisms since a quantization of their presence are used to indicate the potential presence of pathogens in foods. It is believed by some investigators that the higher the numbers of coliforms, the greater the possibility of pathogenic organisms being present. This indicator/pathogenic relationship however, is scientifically debatable and by no means accepted unanimously by the scientific community (Yannick *et al.*, 2013).

Coliforms do not necessarily indicate contamination from a fecal source, in the sense of implying immediate contact with the feces. The presence of large numbers in a processed food indicates that the opportunity of proliferation might have occurred, which could also have allowed multiplication of *Salmonella,* Staphylococci, etc, (Refai, 1979).

Coliforms (certain strains) can also produce illness in man, although meat has not been demonstrated as vector (Brown, 1982).

The finding of *Escherichia coli* higher than 10^2 cfu/g indicates dangerous contamination of food.

Maximum limit for the coliforms according to the EU Microbiological standards of cut meat and retail sale and further processing is 5×10^3/g (Anon., 2003).

2.2.2.3 Spore formers

Spore formers are of two types viz. aerobic spore formers e.g. *Bacillus spp.* such as *Bacillus cereus, B. subtilis* etc. and anaerobic spore formers e.g. Clostridia such as *Clostridium botulinum* type A and B, *Cl. perfringens* etc. (Leistner, 1985).

Small number of *B. cereus* may be found in meat and poultry. Between 1960 and 1968, meat or meat products were implicated in majority of food poisoning out breaks in Hungary (Roberts, 1982).

Cl. perfringens type A is commonly found in meat, poultry and their products. The spores have various degrees of heat resistance ranging from a few minutes to several hours at 100^{o}c; both the heat resistant and heat sensitive strains have been implicated in food poisoning (Sutton and Hobbs, 1968).

2.2.2.4 *Salmonella*

Salmonella in red and white meat is a worldwide problem. Food borne *Salmonella* infection results from the ingestion of large numbers of the organism, which then multiply within the small intestine. Almost all members of the *Salmonella* genus are potentially pathogenic. *Salmonella spp* are common inhabitants of the intestinal tracts of many animals, especially cattle and during slaughter and dressing processes, they can easily contaminate food via fecal contamination (Roberts, 1982).

Less than 1 to large numbers in foods have been implicated in outbreaks. Hence the presence of *Salmonella* at any level in meats is objectionable (Bachhil and Jaiswal, 1988). The risk of *Salmonella* contamination to other foods and subsequent multiplication remains, even when the particular food in question is unable to support the growth. It is therefore undesirable in meat. Although one or few typhoid organisms are found to be sufficient to cause illness in human, it is believed that much higher number are required to cause food poisoning incidences (Corry, 1976).

When referred to EU Microbiological standards for cut meat and retail sale *Salmonella* should not be detected in 1 gram (Anon., 2003). The majority of the meat borne *Salmonella* incidences has been due to the live animal providing meat, and some cases due to under cooking of contaminated meat leading to survival of pathogens. *Salmonella* can reach food from animal excreta at time of slaughter, from human excreta or from water polluted by animal or human sewage. They are brought into kitchen in raw meat and may be transferred to cooked foods via hands, surfaces, utensils and other equipment (Roberts, 1982).

2.2.2.5 *Staphylococcus aureus*

Meat is contaminated with *Staphylococcus aureus* by handling and by sneezing or coughing. Minute amounts of toxin will cause illness, which starts within 1-8 h of eating poisoned food. It does not produce off-odors or spoilage so it cannot be easily checked (Narasimha Rao and Heinz, 1991).

Counts of 10^5/g or less wouldn't be expected to result in enterotoxin production. Minor and have shown that counts must be 10^7-10^8/g for detectable enterotoxin production. The greatest amount is produced at the optimum temperature for growth i.e. 35-37 ^oC. 5×10^3/g is the maximum limit for *S. aureus* on EU Microbiological standards of cut meat and retail sale (Anon., 2003).

2.2.2.6 *Clostridium botulinum.*

As clostridia are part of the normal intestinal flora of animals there is a possibility that *Cl. Botulinum* may be present. Human botulism is almost invariably caused by food which has been inadequately preserved, stored for some time and then consumed cold or without sufficient heating (Roberts, 1982).

Botulism, the most serious form of food poisoning, results from consuming food containing toxin of *Clostridium botulinum* Types A, B, E and F are the main causes in man. The spores, apart from type E are heat resistant and can withstand cooking procedures apart from steam under pressure. The toxins however, can be easily destroyed by heating (Hersom and Hulland, 1980).

2.2.2.7 *Listeria monocytogenes*

Listeriosis occurs mainly in pregnant women, neonates, immune-suppressed patients and the elderly. The causative agent *Listeria monocytogenes* has been isolated from meat processing facilities including soil, sewerage, silage and raw meats. It is excreted on animal faeces. The presence of this pathogen on raw foods is likely to be unavoidable. The organism can grow at pH 4.6-9.6. It can grow in aerobic, micro aerophilic and anaerobic conditions and in the presence of CO_2 (Bobbitt, 2002).

2.2.2.8 Yeasts and molds

They often manifest themselves in foods of low pH, low moisture, high salt or sugar content and can utilize organic acids, proteins and lipids. They spoil by causing off-color and flavor in meat products (Refai, 1979).

If insufficient oxygen is present, they use acid in the food and so increase the pH Current evidence suggests that mycotoxins do not present a major health hazard (Shapton and Shapton, 1991).

2.2.2.9 Viruses and Parasites

Among the pathogenic viruses are those causing hepatitis A and gastroenteritis. In the UK, there are approximately 400 cases per year of Hepatitis A caused by viral infection, gastroenteritis cases total approximately 11,000 per year, having increased from approximately 4500 cases per year in the early 1980's. Hepatitis A is of importance in cold meats. However, the fate of viruses present in meat has received little attention (Shapton and Shapton, 1991).

The most important parasites in meat inspection are those transmissible to man by consumption of the flesh of affected animals, while other parasites, though not transmissible to man may render the flesh or organs repugnant and therefore unmarketable e.g. extensive muscular sarcosporidiosis.

The parasites of importance are Nematodes (round worm), Cestodes (tapeworm), *Trematodes* (flukes), Protozoa and Arthopoda or joint footed animals, including flies and linguatula. Control of such infections can be achieved by avoiding unsanitary disposal of human faeces near cattle or swine feeding areas and by proper cooking (Gracey and Collins, 1994).

Frequent consumption of raw or under cooked meat where there is little inspection can lead to the development of trichinosis in the consumer (Roberts, 1982).

2.3 Meat hygiene in Nepal

In Nepal, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses of meat as well as invaluable by-products (Subba, 1996).

In developing countries, a high percentage of animal slaughter takes place in rural areas under very primitive conditions that do not meet even minimal technical and hygienic requirements. Animals are slaughtered in all kinds of places, such as converted buildings or rooms, under the shade of trees, and on open, bare ground. Animals that have been slaughtered on the ground are then hoisted via the gantry so that the carcass can be dressed. When rural slaughtering takes place on relatively small premises, very simple equipment, such as hooks or ropes for hanging animals and chopping blocks for breaking down carcasses, may be available. However, it remains a common practice to dress carcasses on the building floor. Under these conditions, the utilization of animal by-products generally is low or non -existent, since the byproducts are considered a nuisance (Acharya, 2010).

When meat is sold on one or two market days, meat stalls often are crowded, and customers lean on the stall; the meat becomes contaminated through contact with their hands, bank notes, baskets, clothes, and other objects. The behavior of butchers is not always the most appropriate from a hygienic point of view and may contribute to the problem. In urban areas the traditional marketing of meat begins with early morning slaughter and delivery of the unchilled meat to the market place a few hours later. The FAO recommends that in the long term this be improved to a complete "cold chain" system, with the meat being cooled down at the slaughterhouse and then transported in refrigerated trucks to controlled butcher outlets. The development of the meat sector, in particular in the rapidly expanding population centers, will have to move in this direction for both public health and environmental reasons (Siles *et al.*, 1997).

Once the meat leaves the abattoir, its hygienic quality also is influenced by careless and poor handling. Carcasses, quarters, unwashed offal, and other items are placed together on the floor of the market or on dirty concrete or wooden tables in meat shops, increasing the microbiological contamination of the meat (Subba, 2010b).

Slaughtering places are frequently polluted with street dust, garbage, human excreta, animal blood, intestinal contents and dirty effluents and are not protected against dogs, rodents and insects. Meat products produced under such conditions are generally spoiled due to bacterial contamination and may cause food poisoning. Due to lack of meat inspection, meat from diseased or parasite-infected animals has been the source of infections and transferable diseases to humans as well as animals. Besides meat quality is

adversely affected by careless handling conditions in slaughtering places as well as in the meat market or shops (Joshi, 1991).

The butchers of Kathmandu valley utilize sides of roads, banks of rivers, often ground of their house or any available open places for slaughtering animals. In addition, meat is hung all day in unhygienic surroundings. Meat and gut are kept together for sale by vendors. Microorganism e.g. *E. coli, Staphylococcus aureus*, etc can be readily transferred to meat under such conditions, and there is a potential danger of food poisoning or intoxication (Karki, 1995).

65.7% of butchers of Kathmandu valley kept offal disposal container and 68.6% of butchers have dog proof provision on selling counter. Where as 64.9% of butchers do not know about meat borne diseases and 14.29% have refrigeration facility. Primal cuts of meat are transported to shops by rickshaw or in baskets (Joshi and Olesen Hans, 1999).

Several reports have been published on microbiology of meat from different parts of the Nepal with different organism pattern. Karki (1995) studied the bacteriological quality of poultry and buffalo meat of Kathmandu valley. He reported that *E. coli* and *Staphylococcus aureus* were found in all the samples. Other isolates were *Klebsiella oxytoca, Proteus vulgaris, Enterobacter aerogens, Salmonella arizone, Citrobacter diversus, Tautomella ptyseos, Providencia rettgeri, Plesiomonas shigelloides, Pseudomonas aeruginosa, Streptococcus fecalis* and *Bacillus spp.*

Prasai (2000) studied the microbiological pattern of raw meat of Kathmandu valley. He has reported *E. coli* as the most predominant organism. Other isolates were *Staphylococcus aureus, Salmonella spp., Bacillus spp., Pseudomonas aeruginosa*, followed by *Proteus vulgaris, Enterobacter aerogenes, Citrobacter diversus, Klebsiella oxytoca, Streptococcus faecalis, Citrobacter freundii, Providenci arettgeri, Proteus mirabilis* and *Enterobacter cloacae.*

2.4 Pork meat marketing system in Dharan metropolitan city

Market System operates with the suppliers, producers, sellers and buyers. Pig Producers, sellers and buyers are the Direct Market Players, whereas, feed suppliers, financial institutions and equipment suppliers who are not direct players are also part of Pig and

Pork Market Systems. In addition, business enablers, infrastructure providers and business associations are also the part of market system (Personal Communication).

Since, pig/pork market system in Dharan sub-metropolitan city consists of different direct and indirect market players, and each of them influences the market of pigs and pork they have their roles and influences in the pig and pork market value chain (Personal Communication).

The meat shops are dispersed all over the city centering main market area. It is usual for butchers to slaughter clandestinely in early in the morning on open field. Slaughtering sites are usually unhygienic, often unpaved and poorly drained. Carcasses are held in the same areas as those used for slaughtering, often amongst the debris (blood, legs and heads, gut contents) of earlier slaughters. Animals are mistreated using cruel methods such as restraining their legs with tightly bound ropes. The water used for cleaning is often heavily polluted with dung and the same water is forwarded to clean weapons and carcasses before they are transferred to the shop (Adhikari *et al.*, 2012).

The condition of shops where meat is sold does not comply with minimum expected requirements for hygiene and quality. Animals do not undergo any health inspection and the water used for cleaning is dirty. The transportation of pork meat from slaughtering site to meat shop is done with public vehicles which are unsuited to carrying food products. Auto-tempos, Rickshaws, Cars and Hand-boards all are used and meat is carried unwrapped exposing to flies and dust. Shops do not have refrigeration facility to keep meat in chilled condition. Even many shops do not have toileting facility (Personal Communication).

2.5 Total pork consumption and demand in Dharan sub-metropolitan city

The local consumption of pork in households of Dharan sub-metropolitan city is significantly stable. According to Pig Entrepreneur Association Nepal, 2016 the total daily consumption of pork in Dharan sub-metropolitan city comes to be around 4000 kg per day. The demand of local consumption has not decreased for the last two or three years. It was transpired that the local consumption is gradually increasing with growth in population and also with the increased number of pork eater from other community (though there was not any authentic data available on this) – such as Brahmin, Chhetri, and Newar.

Dharan metropolitan city is known as one of the cities where significant numbers of ethinic groups such as from Rai, Limbu, Gurung and Magar ethnicities have settled. Hence, remarkable consumption of pork by these people from the very inception of this city has been noticed. People from other community such as Chhetris, Brahmin and even Newar consume pork in Dharan sub-metropolitan city, though they do not cook it in their houses for social taboos. These people are main consumers of pork momo and pork fried. The Table 2.1 presents an estimated consumption of pork in Dharan metropolitan city (Pig Entrepreneur Association Nepal (2016).

Table 2.1 Daily Consumption of Pork in Dharan metropolitan city

Pork consumed by	volume (kg)	Number of pig (estimated)
Hotels and Restaurants	279	4
Households	3571	51
Total	3850	55

Source: Pig Entrepreneur Association Nepal (2016)

The total demand of pork in Dharan metropolitan city, based on the above table, can be estimated as to be 3850 kg. which can vary depending on seasons and demand. During Dashain, New Year's Day, and winter season the demand is higher which may exceed the volume depicted in the above table and may reach to 75 to 90 pigs –equivalent to 5250 to 6300 kg. The demand is lower during rainy seasons especially in Ashadh and Shrawan which may be far below 3500 kg/day.(CEAPRED/SAMARTH - NMDP Pig Sector Project, 2016).

2.6 Importance of HACCP in quality of Meat

An important priority in meat production is to minimize contamination with pathogenic organisms during slaughter, dressing and subsequent handling of meat. Although microbiological testing of foods is an important tool to ensure safety, such testing has the disadvantages that it normally requires time and it often detects problems only after they occur. A careful analysis of microbiological hazards can be made and an in-house,

effective monitoring system for quality assurance applied (Bolton *et al.*, 2002; Potter and Hotchkins, 1996).

HACCP plays an important role in retaining good microbiological quality and stability of meat. HACCP is basically a statement of a preventive system of controls based on hazard analysis and critical control points. This involves the identification and control over those processing parameters whose loss of control would result in on unacceptable risk to consumers (Frazier and Westhoff, 1997).

With regard to meat production, the HACCP concept systematically identifies potential hazards in the entire chain from animal production to consumption and ranks them according to severity and likely frequency. This covers facilities, equipment and operation and is intended to augment and refine the various codes of manufacturing practice undertaken industry. The procedure is intended to enable management to take preventive rather than depend on intensive testing of the end-products (Bolton *et al.*, 2002; Narasimha Rao and Heinz, 1991).

Part III

3. Materials and methods

3.1 Meat sample collection plan

The Dharan metropolitan city Sub-metropolitan city is divided into 20 wards. Microbiological Quality of marketed meat was analyzed on the basis of domestic consumption and simple uses. About 3850 kg of pork meat/day is consumed in the city.

Pork meat samples were collected from 7 most selling places. Randomly choosen shops from Zero point, machhavaudi, panbari, vrc, railway, bhotepul and ganeshman chowk are sampling sites.

A sample size of 250 g from each place was collected in sterile polythene bags and analyzed within 2 h of collection. The samples were kept inside the sterile polythene plastic bags without touching by the collector.

Generally the samples were collected in the morning time of at 6-7 AM Sample was processed immediately as soon as possible. An ice box was used during the collection to discourage the growth of microorganisms.

3.2 Chain of custody procedures

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

The following procedures were performed in the present study;

- Manual sampling was done.
- Sample containers were made of polythene plastic packages
- 250 g meat samples were taken to comply with the sampling, handling, analysis,storage and preservation requirements.
- Duplicate destructive samples were taken from each sampling sites.

- Information on sample was collected from the seller himself and other relevant information on the site was noted by the collector.
- The sample was transported in a protected condition (in an ice box) to the laboratory within an hour of completion of sampling.
- During analysis, parameters were processed with the prime priority and analyzed immediately.

3.3 Preparation of meat sample

3.3.1 Homogenization

25 gram of meat sample was aseptically transferred into meat mincer (National meat grinder, Model-MK-G10N, Matsusiuta Electric Ind. Company Ltd.) and 225 ml sterile distilled water was also added in the same machine and homogeneous mixture of sample was obtained. Before starting the mincer, it was thoroughly washed with clean water, distilled water and finally cleaned with 70% alcohol (Brown, 1982).

3.3.2 Serial dilution of homogenate

1 ml of homogenized meat aliquot sample was pipetted and serial dilution of homogenate was done. Samples were serially diluted upto 10^{-7} dilution according to KC and Rai, (2000).

3.4 Sample collection from possible contaminating source

Sample from hands of butcher, chopping board and knife was collected from 7 most selling places. Cotton wool swabs of 4 cm length and 1.5 cm thickness were used. Distilled water was used as diluents (Harrigan and McCane, 1979).

3.5 Analysis

3.5.1 Total Plate Count (TPC)

Total plate count was determined by pour plate method according Harrigan and McCane (1979) using plate count agar and distilled water as diluent.

3.5.2 Total coliform

Coliform count was determined by pour plate method according to (Varadaraj, 1993).

3.5.3 *Staphylococcus aureus*

Staphylococcus aureus enumeration was carried out according to Harrigan and McCane (1979). Triple-Sugar Iron Agar test, IMViC test, gelatin liquefaction test, H_2S test, coagulase and catalase test were done for confirmation.

3.5.4 *Shigella* detection

Shigella was detected according to Harrigan and McCane (1979). Similarly, Triple-Sugar Iron Agar test, IMViC test, gelatin liquefaction test, H_2S test, coagulase and catalase test were done for confirmation.

3.5.5 *Salmonella* detection

Salmonella was detected according to the Varadaraj (1993) with some modifications given in Fig. 3.1

20 g grinded meat sample

↓

Pre- enrichment in 80 ml sterile peptone water

↓

Incubation at 37°C for 16-20 h

↓

Enrichment in Selenite F broth for 24 h at 37°C

↓

Serial dilution in distilled water

↓

Culture from serially diluted dilution on Bismuth Sulphite Agar

,

↓

Incubation at 37°C for 24 h

↓

Biochemical testing of colonies in Triple-Sugar Iron Agar test, IMViC test, gelatin liquefaction test, H_2S test, coagulase and catalase test were done for confirmation.

Fig. 3.1 Flow chart for the detection of *Salmonella* species

3.6 Study of sanitary condition of meat shops and personal hygiene

A questionnaire (Appendix A) was prepared to study sanitary condition of meat shops and personal hygiene of butcher. Questionnaire was compiled along with the study of that area. The data related to the sanitary problems of that area around sampling site was collected and situational analysis conducted.

3.7 Data analysis

Of the six parameters analyzed except two (*Salmonella* and *Shigella*) were statistically analyzed. The raw data were statistical processed for significant difference by ANOVA (Two factors without replication) in the computer using Data Analysis feature of Microsoft Office. LSD comparison was done by Fisher's Least Significant Difference (LSD) Test method.

Part IV

4. Results and discussions

Duplicate destructive samples of pork meat having sample size of 250 g from randomly chosen 7 most selling places were collected in sterile polythene bags, placed in ice box and analyzed within 2 hours of collection. Sample was processed immediately as soon as possible. 25 gram of meat sample was aseptically transferred into meat mincer (National meat grinder, Model-MK-G10N, Matsusiuta Electric Ind. Company Ltd.) and 225 ml sterile distilled water was also added in the same machine and homogeneous mixture of sample was obtained. Samples were serially diluted upto 10^{-7} dilution according to KC and Rai (2000). Samples from hands of butcher, chopping block and knife were collected and microbiological analysis was done. Moreover a survey was conducted to determine the sanitary condition of meat shop.

4.1 Microbiological quality of pork meat

Samples of meat, swabs of chopping board, swabs of knife and swabs of hands of the butchers were analyzed for numeration of Total Plate Count (TPC), Total Coliforms (TC), *E.coli*, and *Staphylococcus aureus.*

Findings of microbiological analysis of pork meat samples, swabs of knives, chopping boards and hands of the butcher can be represented in bar graph in log scale as figure 4.1

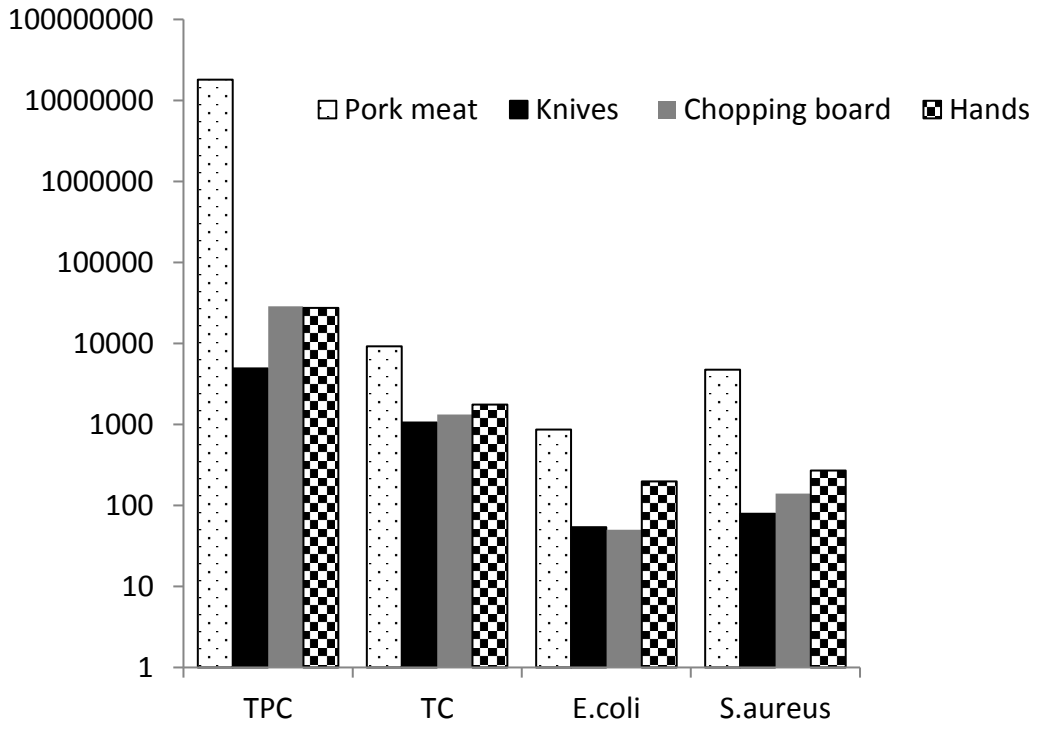

Fig. 4.1 Average microbial counts of pork meat, knives, cutting board and hands of butcher.

Average TPC in meat is found to be 181×10^5 cfu/g. But TPC of chopping board, knives and hands of the butchers is less than TPC of meat averaging 287×10^2, 49×10^2, 274×10^2 cfu/cm^2 respectively.

Similarly fig. 4.1 shows the average value of Total coliform in meat samples, swabs of chopping board, knives and hands of the butchers found to be 918×10^1 cfu/g, 133×10^1 106×10^1 and 176×10^1 cfu/cm^2 respectively.

The average number of *E. coli* present in meat samples, swabs of chopping board, knives and the hand of the butcher. Average *E. coli* of meat samples is 862 cfu/g. Similarly average number of *E. coli* for swabs of chopping board, knives and hands of the butcher was found to be 50, 54, 198 cfu/cm^2 respectively. *S. aureus* of meat also found higher than swab samples. 473×10^1 cfu/g is the average value of *S. aureus* in meat samples. Average

value of *S. aureus* in swabs of chopping board, knife and hands of the butchers was found to be 14×10^1, 80, 271 cfu/cm^2.

From the statistical analysis, ANOVA two factors without replication (Appendix D), no significant difference was found at 5% level of significance among the sampling sites for the parameters TPC, total coliforms, *E. coli* and *S. aureus*. This can be further evidenced by the survey also. Such results can be directly correlated with the sampling sites keeping in mind that sampling sites were found to be in the same condition and same type of atmosphere. The way by which animals are handled, slaughtered and meat sold were observed to be the same. But significant difference (at 5% level of significance) was found among the sample type viz. meat sample and swab samples of knife, chopping block and hands of butchers for the parameters TPC, total coliforms, *E. coli* and *S. aureus.* From the LSD table sample types meat and swabs of knives, meat and swabs of chopping board, meat and swabs of hands were significantly different to each other in terms of TPC, total Coliforms, *E. coli* and *S. aureus*. But swabs of knives, swabs of chopping board and swabs of hands were not significantly different at 5% level of significance level to each other in terms of TPC, total Coliforms, *E. coli* and *S. aureus.*

The present study is matched with Adhikari *et al.,* (2012) that the contamination of pork meat was due to non potable water, unclean utensils, knives, unscientific slaughtering practices and cruel handling methods, besides, environmental contamination and handling of meat in its preparation and sales besides these unscientific storage lack of scientific methods of storage and due to lack of knowledge of microorganisms, many types of microorganisms introduce into the meat.

Taking the reference of microbial standards of Europe and United States (Appendix C) the average total plate count was found to be higher than the inspected German quality meat standards referred for cutting and packaging plant which is less than 5×10^6/g. It was also greater than the Oregon state microbiological standard for fresh meat i.e. 5×10^6/g. The average total coliform count of the analyzed sample was also found beyond the EU microbiological standard of cut meat for retail sale and further processing i.e. 5×10^3/g.

It was revealed that the present study was similar to Adhikari *et al.,* (2012) that is meat and meat products are found to be contaminated due to the exposure of meat to different sources of microbial contamination including contact with hide, viscera, mucous secretion,

hands and clothing of personnel, water used for washing carcass and even air in the processing and storage area. As per Adhikari *et al.* (2012) High numbers of coliforms indicate inadequate cleaning, unsanitary handling and post processing contamination from dirty atmosphere around shops which has also matched with present study.

The present study has shown that the TPC value was higher than the Oregon state microbiological standards value, which indicates that the consumers are at greater risk of being food poisoned. Here the study was also matched Brown, (1982).

Meat sample covers whole microbial load of primary and secondary contamination from the sample taken whereas other sample (swabs) analyzed only contained secondary contamination, so the result can be attributed as such. The possible reasons behind this maybe due to the difference among the cleaning and sanitizing habits. Frequency of cleaning was found to be different among the butchers. Higher number of microbes on meat samples which might be due to other contaminating sources also. The cross contamination from these sources could not be ignored. Selling of intestinal and respiratory tract along with the meat and handling by same man with same cutting knives can spread the *coliforms* and other microbes which was matched with Varadaraj, (1993).

Microbiological analysis showed heavy contamination of knives, chopping blocks and hands. Because of varied sources, the kinds of microorganisms likely to contaminate meat are many. This directly reflects highly polluted and unhygienic condition of meat being sold on local market of Dharan sub-metropolitan city.

4.2 Detection of *Salmonella* and *Shigella* in pork meat, chopping board, knives and hands of butchers

Pork meat samples, swabs of chopping board, knives and hands of the butcher were examined for the presence or absence of *Salmonella* and *Shigella*. Table 4.2 shows the presence or absence of *Salmonella* and *Shigella* in pork meat, chopping board, knives and hands of butchers.

Table 4.2 *Salmonella* and *Shigella* in pork meat, chopping board, knives and hands of butchers

Source	*Salmonella*				*Shigella*			
	Meat	Board	Knives	Hands	Meat	Board	Knives	Hands
A	-	-	-	+	-	-	-	-
B	+	-	-	+	+	+	+	+
C	-	+	-	+	+	+	-	-
D	-	-	-	-	+	-	-	-
E	+	-	+	-	+	+	-	-
F	+	-	-	-	+	-	-	-
G	+	+	-	+	+	+	-	+

+ = Positive, - = Negative

(Note: A=Zero point, B=Machha vaudi, C=Panbari, D=VRC, E=Railway, F=Bhotepul, G=Ganeshman chowk, which are different sample from Dharan metropolitan city).

In case of *Salmonella* two samples(C and G) of chopping board, one sample (E) of Knives and four swab sample (A, B, C and G) of hands of butchers were found to be positive. Out of seven swab samples five samples (A, B, D, G and H) of chopping board, three samples (D, G and J) of Knives and two samples (A and J) of hands were detected for *Shigella.*

It was revealed that the contamination of meat with *salmonella* and *shigella* was done due to use of non potable water, contaminated hands of butchers and utensils which was also matched with Roberts (1982).

The pork meat samples may be contaminated by *salmonella* and *shigella* due to contaminated carcass, slaughtering environment, knives and other equipments which is also described by Duggan (2010) and Bonardi (2003).

Due to lack of farm to fork food safety practices in Nepal, illness causing bacteria like *Salmonella* and *Shigella* were found in most of the meat and swab samples of chopping board, knife and hands of the butchers. This analysis directly reflects highly polluted and unhygienic condition of meat being sold on local market of Dharan sub-metropolitan city.

There is lack of properly managed and scientific slaughter houses. Due to lack of interest and monitoring by the concern authorities and lack of public awareness, meat processing is being done on the floor, there is no practice of postmortem and ante mortem inspection of animals and meat, sterilization of equipment and wearing of clean apron and fencing the shop are rarely done. So Good Manufacturing and Good Hygiene Practice are the current demand in slaughtering, selling, production and marketing of pork meat in Dharan sub-metropolitan city.

4.3 Survey on the sanitary condition of meat shop

From the survey with the help of questionnaire (Appendix A) of the entire pork meat shops and interview with butchers suggest about the unhygienic and unscientific method of handling, lack of sanitation and knowledge of microorganisms resulting in higher number of contamination. The detailed survey finding is given in Appendix B.

4.3.1 Sanitary condition of meat/shop

It was found that 85% of the meat sellers control the flies manually. 15% was found using chemical to get rid of the flies. 42.85 % of the butchers clean the shop daily while 57.14 % of the butchers clean their shop only 2-4 times a week. Further it was found that 76.67% of the meat handlers sanitize meat processing equipment before each use.

It was found that 57.14 % of the shops used water for cleaning, 28.58 % used soap or detergent powder as sanitizing agent. Few (14.28%) used cloth for the cleaning purpose. 51.42 % butchers did not use apron.

From the survey, sanitary condition of the shop was found satisfactory (54.28%). 20 % of the shops were found dirty and 25.71% of the shops were found and observed to be well cleaned.

4.3.2 Selling condition

It was found that 28.57% of the shops have metal wire fence around shop while 71.42% of the shops did not have metal wire fencing to protect the meat from dogs and rodents. Only 28.57% sold meat on cemented platform. 42.85% sold on the wooden table while 8.57% used carpet and 20% used tin plate for serving meat in the shop.

4.3.3 Storage of meat/leftovers

When asked about leftovers, it was learnt that 68.57% kept the meat in refrigeration while 25.71% said they would sell the meat following day and only 5.71% said they dump the leftovers. 31.42 % shops did not use refrigerator for leftovers while rest 68.75 % used refrigerator for storing the leftovers.

4.3.4 Knowledge about Zoonoses

85.71% butchers responded that they did not examine the animal for diseases before slaughter. 62.85% of the butchers were unaware of zoonoses while 37.14 % had the knowledge that meat was a prominent source of disease**.**

4.3.5 Knowledge about Acts and Regulations

Survey showed that 8.57 % meat personnel were found to be familiar and 91.43 % denied having any idea about the Slaughterhouse and Meat inspection Act. Only 5.71% of the butchers have slaughter house.

4.3.6 Facility found in shops

Rickshaw/city safari (85.71%) was found to be most prominent transportation vehicle and 14.28% used self carrying option. 54.28 % of the shops had nearly located toilet whereas 45.71 % shops had no facility of toilets. The entire 35 butcher utilized tap/tank water for the further processing of meat.

The survey showed that the hygiene around meat shop to be quite unsatisfactory. The contamination of meat by equipment begins with the slaughtering the animal. During the slaughtering operation, the equipment used comes in contact with maximum of animal surfaces. When the animal is cut and served to consumer equipments such as knives, cutting blocks and the seller's hand are the main sources for the cross contamination of the

meat. The cleanliness of the utensils, knife and other contact surfaces therefore are equally responsible for the poor hygiene quality of the marketed meat in Dharan metropolitan city. Survey findings were similar to the study of Joshi and Olesen (1999) and Adhikari (2012).

Part V

5. Conclusions and recommendations

5.1 Conclusion

Hygiene quality of pork meat marketed in Dharan sub-metropolitan city was assessed by enumerating the microbial load of the meat samples and by questionnaire survey on shops and butchers. Randomly chosen seven places were used to take meat samples.

Following conclusions can be drawn from the research work:

1. All the pork meat samples were found to contain higher microbial load than prescribed standards of Europe and United States. The pork meat, therefore, is not bacteriologically sound. However, the outbreak of zoonotic diseases have not been recorded so far due to intense cooking practices in locality.
2. Except three meat samples all were found to contain *Salmonella* and only one sample of meat was free from *Shigella.*
3. Hygienic condition of meat shops and knowledge of GMP and GHP in meat production are poor.

5.2 Recommendations

The pork meat samples of Dharan metropolitan city were found to contain high counts of micro organisms. The hygiene quality was unsatisfactory. The findings imply that people of these areas need to be careful about the quality.

To improve the bacteriological quality of the raw meat some well-known, simple techniques that can be recommended are:

1. Slaughter of animals in properly constructed hygienic surroundings by improved and humane method.
2. Proper zoning plans with provisions of clean water places to dispose of wastes blood, gut content, bones etc.
3. Animal slaughterhouse and meat inspection act and other acts concerning meat commodity should be fairly implemented for better quality and disease free meat, and for standardization of meat handlers and their facility.

4. Regular checks on meat quality by concerned authority need to be strictly implemented for public health protection.
5. Training programs on humane method should be conducted to upgrade the small firms as well as butchers ensuring that they are more aware of their responsibilities to the public.
6. Consumer awareness campaign should be arranged to promote the good products.

Part VI

6. Summary

The raw pork meat sampled from seven different places was examined for the enumeration of total plate count, total Coliforms, *E. coli* and *Staphylococcus aureus. Salmonella* and *Shigella* were also checked for their presence and absence.

Swab samples of Chopping boards, Knives and Palms of Butchers were also studied for the above mentioned micro organisms.

Average value for TPC of meat sample was found to 181×10^5 cfu/g. The average coliform, *E. coli* and *Staphylococcus aureus* counts were 918×10^1, 862 and 473×10^1 cfu/g respectively. Except three samples, all samples were found to be infected with *Salmonella* where as six out of seven samples were found to be *Shigella* positive.

The average value for total plate count of Chopping board, knives and palms of butchers were found to be 287×10^2, 494×10^1 and 274×10^2 cfu/cm^2 respectively. The average Coliform, *E. coli* and *Staphylococcus aureus* counts of chopping board were found to be 133×10^1, 50 and 14×10^1 cfu/cm^2. The average Coliform, *E. coli* and *Staphylococcus aureus* counts of knives were found to be 106×10^1, 54 and 80 cfu/cm^2. The average Coliform, *E. coli* and *Staphylococcus aureus* counts of the palms of butchers were found to be 176×10^1, 198 and 271 cfu/cm^2. Five swabs of chopping board, six swabs of Knives and three swabs of hands were *Salmonella* free. Out of seven swab samples four samples of chopping board, one samples of Knives and two samples of hands were detected for *Shigella*. Thus, from the work it is observed that bacterial contamination of the meat samples is dependent on the micro flora of possible contaminating sources. The examined contaminating source also showed heavy population of micro organisms.

From the survey it was cleared that pork are slaughtered unhygienically and unscientifically. The methods of slaughtering animal and serving meat need to be upgraded. Personal hygiene of the butchers needs high improvements through awareness campaign. Waste disposal places should be clearly allocated. There is need of strict enforcement of Animal slaughterhouse and meat inspection act and education about sanitation.

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Appendices

Appendix A

Questionnaire used for Survey of Sanitary condition of meat shop

Name: Address:

Signature: Sample Code:

1. How do you control flies in your shop?
    a. Chemically
    b. Manually
    c. Do nothing
2. How often do you clean the shop on a week?
    a. Seven
    b. Two - four
    c. One
    d. Zero
3. Is equipment used to process meat cleaned and/or sanitized before each use?
    a. Yes
    b. No
4. What do you use while cleaning?
    a. Water
    b. Soap/detergent powder
    c. cloth
5. Do you use apron while processing?
    a. Yes
    b. No
6. How is the sanitary condition in the shop?
    a. well cleaned
    b. dirty
    c. satisfactory
7. Do you have metal wire fence around the shop?
    a. Yes

b. No

8. Where do you place the meat in the shop?
    a. Cemented place
    b. Wooden plate
    c. Tin plate
    d. Carpet
9. What is done with the leftovers?
    a. Refrigeration
    b. Selling next day
    c. Left as it is
    d. Dispose off
10. Do you have refrigerator in shop?
    a. Yes
    b. No
11. Is the slaughtering animal examined before killing?
    a. Yes
    b. No
12. Do you have knowledge of zoonoses?
    a. Yes
    b. No
13. Are you familiar with the Slaughterhouse and Meat Inspection act?
    a. Yes
    b. No
14. Do you have slaughterhouse?
    a. Yes
    b. No
15. How do you transport meat from the slaughtering place?
    a. Car
    b. Handcart
    c. Rickshaw
    d. Carrying
16. Is there toilet near the shop?
    a. Yes

b. No

17. From which source do you use water?
    a. Tap water
    b. River water

Appendix B

Table B.1 Detail output of Survey

Survey was conducted in 35 pork meat shops of Dharan sub-metropolitan city to find out the detail about the sanitary and hygienic condition of meat shops.

Total no of respondents = 35

Survey question number	No. of Respondents			
	A	B	c	D
1	6	29	-	-
2	15	20	-	-
3	27	8	-	-
4	20	10	5	-
5	18	17	-	-
6	10	7	18	-
7	10	25	-	-
8	10	14	7	4
9	24	8	-	3
10	24	11	-	-
11	6	29	-	-
12	13	22	-	-
13	4	31	-	-
14	3	32	-	-
15	-	-	30	5
16	19	16	-	-
17	35	-	-	-

Appendix C

Microbiological Standards

1. Guidelines for Total Plate Count in Meat and Meat Products

Product	TPC Max
Fresh Meat (Cut and Packaging meat)	5×log 6/sq.cm or g
Separated Meat	5×log 6/ g

2. Inspected German Quality Meat

≤ log 4/ g or sq.cm. in freshly slaughtered meat

≤ 5×log6/g or sq. cm. in cutting and packaging plant

3. Danish Quality Assurance Warranty

≤ log 4/ sq. cm. in freshly slaughtered meat

4. EU microbiological standards of cut meat for retail sale and further processing

Bacteria	M	m
Coliform bacteria n=5, c=2	5 × log 3/g	5 × log 2/g
S. aureus n=5, c=2	5 × log 3/g	5 × log 2/g
Salmonella n=5, c=0	not detectable in 1g	

4. Oregon State Microbiological Standard

Total Plate Count	max. 5 × 106/g
E. Coli	max. 50/g

(Note: M = maximum limit; beyond which meat is not acceptable, and applies the following:

M = 10m while counting in solid medium

M = 30m while counting in liquid medium

m = limit, at and under which meat is acceptable

n = number of replicates

c = number of replicates, the count of which lies between m and M.)

(Source: Anon., 2003)

Appendix D

Meat samples, swab samples of chopping board, knives and hands of the butcher which were taken from pork meat shop of 7 different places of Dharan sub-metropolitan city were analyzed and results of microbial count can be represented in following tables.

Table D.1 TPC in Pork Meat, Chopping board, knives and hands of butchers

Unit cfu/g

Source/Site	Meat	Chopping board	knives	hands of butchers
A	165×10^4	120×10^1	60×10^2	68×10^3
B	75×10^6	71×10^2	25×10^2	42×10^3
C	49×10^5	58×10^3	18×10^2	31×10^3
D	50×10^5	21×10^2	45×10^2	81×10^2
E	79×10^5	45×10^3	62×10^2	32×10^3
F	33×10^5	91×10^2	91×10^2	21×10^2
G	29×10^6	79×10^3	46×10^2	86×10^2
Average	181×10^5	287×10^2	49×10^2	274×10^2

Here, TPC=Total Plate Count, A=Zero point, B=Machha vaudi, C=Panbari, D=VRC, E=Railway, F=Bhotepul, G=Ganeshman chowk

Table D.2 TC in pork meat, swabs of chopping board, knives and hands of butchers

Source/Site	Meat	Chopping board	knives	hands of butchers
A	76×10^2	71×10^1	20×10^2	38×10^2
B	24×10^2	52×10^1	25×10^1	25×10^2
C	91×10^1	35×10^2	36×10^1	48×10^1
D	42×10^2	120	61×10^1	68×10^1
E	53×10^2	78×10^1	58×10^1	40×10^2
F	39×10^2	25×10^1	35×10^2	180
G	40×10^3	31×10^2	120	65×10^1
Average	918×10^1	133×10^1	106×10^1	176×10^1

Here, TC=Total Coliform, A=Zero point, B=Machha vaudi, C=Panbari, D=VRC, E=Railway, F=Bhotepul, G=Ganeshman chowk

Table D.3 *E.coli* in Pork Meat, Chopping board, knives and hands of butchers

Source/Site	Meat	Chopping board	knives	hands of butchers
A	52	ND	41	21×10^1
B	170	ND	32	62×10^1
C	170	20	ND	35×10^1
D	160	30	120	ND
E	38×10^1	20×10^1	35	21×10^1
F	20×10^1	ND	150	ND
G	49×10^2	100	ND	ND
Average	862	50	54	198

Here, ND=Not Detected, A=Zero point, B=Machhavaudi, C=Panbari, D=VRC, E=Railway, F=Bhotepul, G=Ganeshman chowk

Table D.4 *S.aureus* in Pork Meat, Chopping board, knives and hands of butchers

Source/Site	Meat	Chopping board	knives	hands of butchers
A	48×10^1	30×10^1	ND	ND
B	45×10^2	150	ND	35×10^1
C	68×10^2	20×10^1	25×10^1	75×10^1
D	63×10^2	ND	ND	20×10^1
E	46×10^2	130	28	60×10^1
F	53×10^2	ND	28×10^1	ND
G	51×10^2	20×10^1	ND	ND
Average	473×10^1	14×10^1	80	271

Here, ND=Not Detected, A=Zero point, B=Machha vaudi, C=Panbari, D=VRC, E=Railway, F=Bhotepul, G=Ganeshman chowk

Appendix E

Table E.1 ANOVA Two factor without replication for TPC

Source of variation	SS	df	MS	vr.	Fpr.
Sample	5.152×10^{15}	3	1.717×10^{15}	10.30	<.001
Residual	1.334×10^{16}	80	1.668×10^{14}		
Total	1.850×10^{16}	83			

Table E.2 LSD to analyze difference between average values in terms of TPC

LSD=7931573.9

Sample type	Average	Difference of Average	Comments
Meat	18100000	M-K =18095060	>LSD
Swabs of Knives	4940	M-C = 18071300	>LSD
Swabs of Chopping Cart	28700	M-H = 18072600	>LSD
Swabs of Hands	27400	C-K =23760	<LSD
		C-H = 1300	<LSD
		H-K = 22460	<LSD

Here,

M = Meat,

K = Swabs of Knives,

C = Swabs of Chopping cart and

H = Swabs of Hands

Table E.3 ANOVA Two factor without replication for Total Coliforms

Source of varriation	SS	df	MS	vr.	Fpr.
Sample	6.077×10^{8}	3	2.026×10^{8}	6.07	<.001
Residual	2.672×10^{9}	80	3.340×10^{7}		
Total	3.279×10^{9}	83			

Table E.4 LSD to analyze difference between average values in terms of total Coliforms

LSD=3549.1

Sample type	Average	Difference of Average	Comments
Meat	9180	M-K =8120	>LSD
Swabs of Knives	1060	M-C = 7850	>LSD
Swabs of Chopping Cart	1330	M-H = 7420	>LSD
Swabs of Hands	1760	C-K = 270	<LSD
		H-**C** = 430	<LSD
		H-K = 730	<LSD

Here,

M = Meat,

K = Swabs of Knives,

C = Swabs of Chopping cart and

H = Swabs of Hands

Table E.5 ANOVA Two factor without replication for *E. coli*

Source of varriation	SS	df	MS	vr.	Fpr.
Sample	9642861	3	3214287	3.42	0.021
Residual	75261299	80	940766		
Total	84904159	83			

Table E.6 LSD to analyze difference between average values in terms of *E. coli*

LSD = 595.7

Sample type	Average	Difference of Average	Comments
Meat	862	M-K =808	>LSD
Swabs of Knives	54	M-C =812	>LSD
Swabs of Chopping Cart	50	M-H = 664	>LSD
Swabs of Hands	198	K-C = 4	<LSD
		H-C = 148	<LSD
		H-K = 144	<LSD

Here,

M = Meat,

K = Swabs of Knives,

C = Swabs of Chopping cart and

H = Swabs of Hands

Table E.7 ANOVA Two factor without replication for *S. aureus*

Source of variation	SS	df	MS	vr.	Fpr.
Sample	328292831	3	109430944	93.50	<.001
Residual	93631325	80	1170392		
Total	421924156	83			

Table E.8 LSD to analyze difference between average values in terms of *S. aureus*

LSD = 664.4

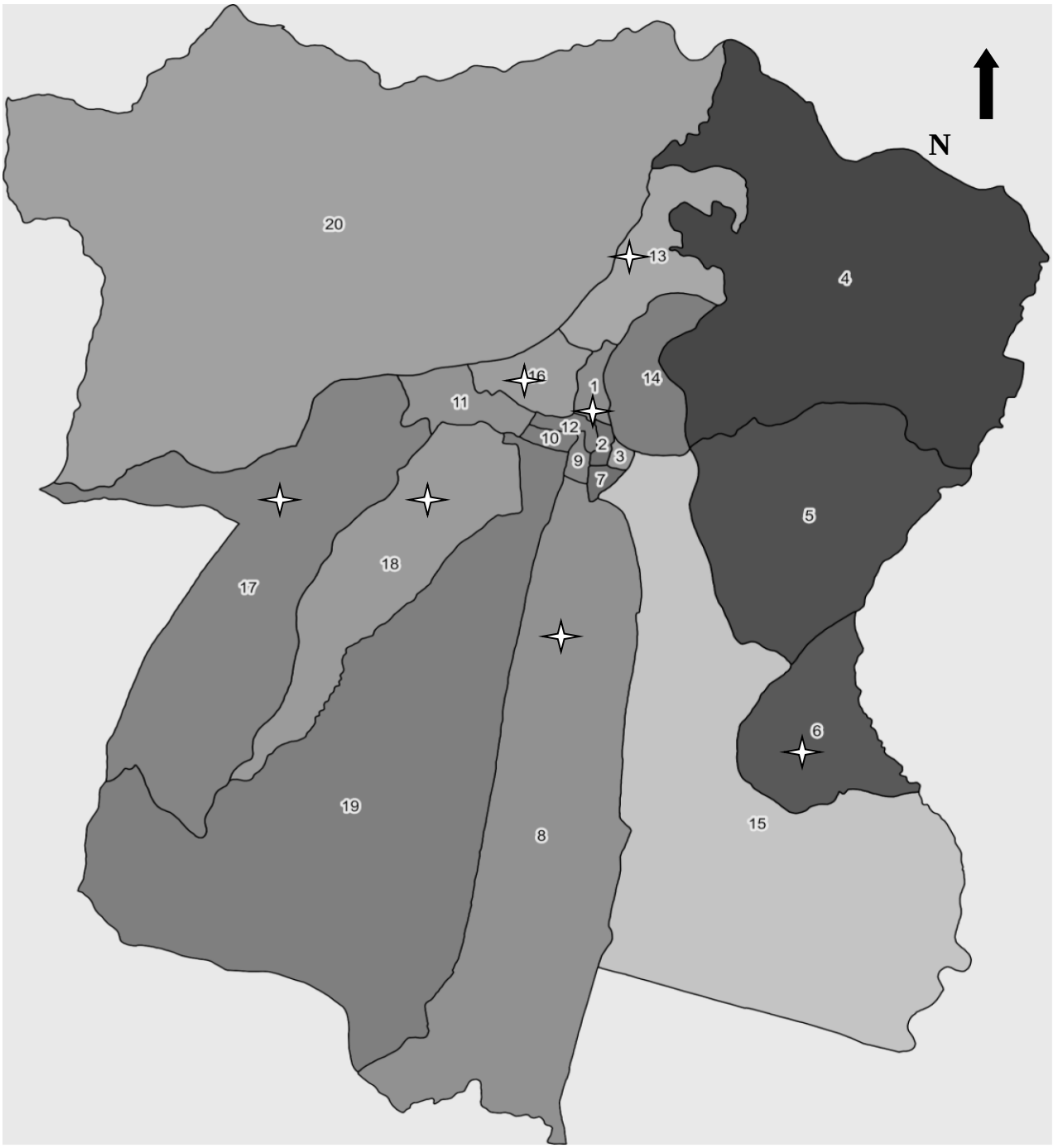
Sample type	Average	Difference of Average	Comments
Meat	4730	M-K =4651	>LSD
Swabs of Knives	80	M-C = 4590	>LSD
Swabs of Chopping Cart	140	M-H = 4459	>LSD
Swabs of Hands	271	C-K = 61	<LSD
		H-C = 131	<LSD
		H-K = 192	<LSD

Table E.9 Average microbial counts of pork meat, knife, chopping board and hands of the butcher

Sample	TPC	TC	*E.coli*	*S.aureus*
Pork meat	181×10^5	918×10^1	862	473×10^1
Knives	49×10^2	106×10^1	54	80
Chopping board	287×10^2	133×10^1	50	140
Hands	274×10^2	176×10^1	198	271

Appendix F

Map of sampling site



= Sampling site in Dharan sub-metropolitan city

Appendix G

Plates

Plate F.1: Busy Pork Meat shop

Plate F.2 Meat sample collection