EVALUATION OF HYGIENIC QUALITY OF RAW MEAT (MUTTON AND CHICKEN) AND CHARACTERIZATION OF ISOLATED PATHOGENS

By

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(J-14-MV-381)

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Division of Veterinary Public Health and Epidemiology
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CERTIFICATE-I

This is to certify that the thesis entitled **"Evaluation of hygienic quality of raw meat (mutton and chicken) and characterization of isolated pathogens"** submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** in **Veterinary Public Health and Epidemiology** to the Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu is record of bonafide research, carried out by **Ms. Huma Kousar Zargar**, Registration No. **J-14-MV-381** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that help or assistance received during the course of the thesis investigations have been duly acknowledged.

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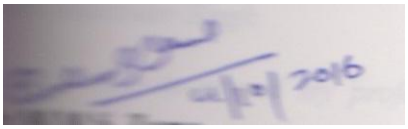

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Date:
Place: Jammu

Signature of the Student

ABSTRACT

Title of the Thesis	**:**	**EVALUATION OF HYGIENIC QUALITY OF RAW MEAT (MUTTON AND CHICKEN) AND CHARACTERIZATION OF ISOLATED PATHOGENS**
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The present study was undertaken to evaluate the hygienic quality of raw mutton and chicken along with characterization of the isolated pathogens. A total of 145 samples (mutton-75, chicken-70) were collected from different locations of Jammu city. Mean ± SE values (log_{10}cfu/g) of standard plate count, *E. coli count* and *Staphylococcus aureus* count in mutton samples were 6.12±0.08, 3.30±0.55 and 4.08±0.15, respectively and the counts in chicken samples were 6.17±0.05, 3.99±0.13 and 4.16±0.09, respectively. The counts were higher than the prescribed microbiological standards (BIS, 1995; FSSAI, 2011) thereby indicating gross unhygienic status. Out of 145 samples, 52 (35.9%) samples revealed *E. coli* whereas 49 (33.7%) samples yielded *S. aureus*; *Campylobacter* were detected from 21 samples (14.4%). Out of 21 isolates of Campylobacter, 16(76.1%) were *C. jejuni* and 5(23.8%) were *C. coli.* The *E. coli* isolates were found to be most sensitive to ciprofloxacin (88.46%) followed by polymixin B (78.84%) and amoxycillin (75%). Isolates of *S. aureus* were found to be most sensitive to ciprofloxacin (91.84%) followed by amikacin (91.83%), polymixin B (83.67%) and chloramphenicol (73.46%) while most (100%) resistant to nalidixic acid. *Campylobacter* spp. were found to be most sensitive to nalidixic acid (100%) and most resistant to cephalothin (100%). Based on hygienic evaluation and presence of pathogens in mutton and chicken meat being sold in the markets of Jammu city necessitates remedial measures including creating awareness about hygienic practices during slaughtering and subsequent handling of meat.

Key words: Hygienic evaluation, chicken, mutton, *E. coli*, *S. aureus*, *Campylobacter* spp.

Signature of Major Advisor **Signature of the Student**

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ABBREVIATIONS AND SYMBOLS USED

APC	Aerobic Plate Count
APHA	American Public Health Association
BIS	Bureau of Indian Standard
BHI	Brain Heart Infusion
BPA	Baird Parker Agar
CDC	Centre of Disease Control and Prevention
Cfu/cm^2	Colony forming unit per square centimeter
Cfu/gm	Colony forming unit per gram
Cm	Centimeter
C	Degree celsuis
C.coli	*Campylobacter coli*
C.jejuni	*Campylobacter jejuni*
ECC	*E. coli* count
EPEC	Enteropathic *E. coli*
ETEC	Enterotoxigenic *E. coli*
EHEC	Enterohemorrhagic *Escherichia coli*
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tera acetic acid
EMB	Eosin Methylene blue
FSSAI	Food Safety and Standard Authority of India
µg	Micro gram
µl	Micro litre
i.e.	That is
e.g.	*exampli gratia* (for example)
et al.	*et alii/ Alia* (and other people)
etc.	*et cetera* (and other things)
Fig	Figure
gm	Gram
hrs	Hours
HUS	Haemorrhagic Uraemic Syndrome

HACCP	Hazard Analysis Critical Control Point
DNase	Deoxyribonuclease
ICMSF	International Commission on Microbial Specification for food
Lbs	Pounds
MHA	Mueller Hinton Agar
Mins	Minutes
ml	Milli-liter
mM	Mili molar
MSA	Mannital Salt Agar
MR	Methyl Reduction
n	Number
ND	Not detected
NSS	Normal saline solution
%	Percent
±	Plus minus
SAC	*Staphylococcus aureus* count
S.aureus	*Staphylococcus aureus*
SPC	Standard plate count
SSSS	*Staphylococcal* Skin Scalded Syndrome.
SE	Standard Error
SPSS	Statistical Package For Social Sciences
STEC	Shiga Toxin producing *E.coli*
TCS	Thermophillic *Campylobacter* spp.
WHO	World health organization
VP	Voges- Proskauer

Chapter-1

Introduction

INTRODUCTION

WHO defines food borne diseases as, "Any disease of an infectious or toxic nature caused by consumption of food or water" (WHO, 1997). Food-borne infections are a major public health concern that encompasses a wide spectrum of gastroenteric illnesses caused by viral, bacterial, parasitic or chemical contamination of food (EFSA, 2009). There are over 200 known microbial, chemical or physical agents that can cause illness when ingested (Acheson, 1999). In the United States, an estimated 46 million foodborne infections occur each year, along with 250,000 hospitizations and 3,000 deaths (Scallan *et al.,* 2011) and the data regarding developing countries including India are lacking because of deficiency of organized food-borne disease surveillance programme. Foodborne pathogens of most significance are bacterial pathogens and are: *Escherichia coli, Staphylococcus aureus, Salmonella* spp.*, Campylobacter jejuni, Yersinia enterocolitica, Listeria monocytogenes, Bacillus cereus, Shigella spp., Vibrio parahaemolytics* etc. (Norrung *et al*., 2009; Borah *et al*., 1992). The transmission of these micro-organisms occurs mainly through contaminated food and water and through faeco-oral route or improper storage and handling.

Meat is an important edible postmortem component originating from the animals that are used as food. The increasing demand for animal proteins, like meat and meat products, has increased the load of slaughterhouses resulting in inadequate attention being paid to the hygienic aspect of meat production. The muscle tissues obtained from the healthy animal slaughter is usually sterile although, freshly slaughtered animals may harbor few bacteria. However, during the process of converting live food animal into meat, microbial contamination of carcass surface is unavoidable. During the process of dehiding, evisceration, cutting process, packaging, etc. meat is exposed to various environmental contaminants. As a result, it gets contaminated with various bacteria which induce the spoilage change in the meat. The contaminants may also be present due to diseased animals, unhygienic environments (polluted water, air etc.), unhygienic

butchers habits/processing methods, faulty slaughtering procedure, post slaughter handling and storage etc. (Mawia *et al*., 2012). The presence of a meat inspection system examines grossly apparent abnormalities during the ante-mortem and post mortem examination, but does not recognize complex microbial contamination, which could later precipitate major public health hazards and economic losses due to food poisoning and spoilage of meat. In India temperature and humidity are ideal for growth and survival of micro-organisms (Chaubey *et al*., 2004). Hot climate and lack of proper storage facilities render meat vulnerable to spoilage, thus posing risk to consumers.

Raw meat may harbour many important pathogenic microbes such as *E. coli, S. aureus, Salmonella* spp., *Campylobacter jejuni, Listeria monocytogenes* etc. making such a meat a risk for human health (Mead *et al.,* 1999). *E. coli* is the most common aerobic organism in the gastrointestinal track of man and many other animals and is considered as an index of faecal pollution which has wide implications in food microbiology to monitor sanitary conditions in food establishments. An outbreak of *E. coli* occurred in 1992 where 144 were hospitalised due to consumption of hamburger from many states of New York, CDC reported *E. coli*157:O7 outbreak in U.S. in 2009 (CDC, 2009). In 2011, *E. coli* O104:H4 outbreak in Europe where 4321cases were reported (CDC, 2009).

S. aureus is a major cause of food poisoning in man as well as range of extraintestinal infections. Enterotoxins produced by *S. aureus* are responsible for the symptoms of *Staphylococcal* food poisoning and may have role in the pathogenicity of some other *Staphylococcal* disease. Humans and animals are the primary reservoirs of *S. aureus* which may harbor *S. aureus* in the nasal passages, throat, on hair and skin (CDC, 2003) from where the contamination of meat may occur. Further, the pathogen has emerged as important nosocomial pathogen throughout the world.

Campylobacter has risen rapidly in importance from obscurity to a significance approaching or even exceeding *Salmonella*. Of the many species under *Campylobacter* spp., *C. jejuni* and *C. coli* are the most common pathogens responsible for majority of human enteritis cases (Allos and Blaser, 1995; Frost, 2001). *Campylobacters* have been isolated from all common food animals and birds (Blaser *et al*., 1984). Poultry is the main reservoir of *C. jejuni* but do not show signs of clinical disease and act as a source of

infection for healthy animals and human beings. Poultry meat is an emerging field for disease transmission because it is easily available and has good digestibility. Poultry meat is more popular in the consumer markets in India because of its acceptance by people (Yashoda *et al.,* 2001). The presence of pathogenic and spoilage micro-organisms in poultry meat and its by-products remains a significant concern for suppliers, consumers and public health officials worldwide. Improper methods of production, storage, handling and preparation have resulted in many recognized international outbreaks.Thus, foods of animal origin need to be closely monitored during processing as well as during handling processing and distribution (Anon, 2002).

Although most *Campylobacter* cases are sporadic, outbreaks do occur. Outbreaks have most commonly been associated with the consumption of raw 2003; untreated or contaminated water (Frost *et al*., 2002; Smith *et al*., 2006). Several water-borne outbreaks have been reported from Finland (Kuusi *et al*., 2005) and *Campylobacter* accounted for three of 14 water-borne outbreaks between 1998 and 1999 (Miettinen *et al*., 2001). The most recent water-borne outbreak, caused by leakage of sewage into the water supply system, was in December 2007 in Nokia Finland, affecting approximately 8000 cases (EFSA, 2007). In 2006, there were in all 400 reported *Campylobacter* outbreaks in the European Union, affecting 1304 persons from 17 different countries (EFSA, 2007). The majority were household outbreaks (71%), followed by restaurants and canteens or workplace catering outbreaks.

Despite this, very little information is available on the true level of exposure of specific populations to potential hazards, particularly in the case of bacterial diseases transmitted by consumption of meat and meat products. Attempts to quantify human health risks consequent to exposure to food-borne hazards largely rely on extrapolation, to the population at large, of information gained from individual disease outbreak investigations. Additionally another risk factor for an increase in bacterial resistance is an increased use of antibiotics for therapy and prevention of bacterial infections as well as growth promoters (Bogaard *et al*., 1997). Treatment and control of food- borne infections is increasingly becoming difficult due to indiscriminate use of antibiotics as therapeutics and prophylactic agents as well as growth promoters among animals. Emerging drug

resistance in the foodborne bacterial isolates is a great public health concern thus warranting the careful use of antimicrobial agents, especially in veterinary medicine (Caprioli *et al*., 2000). The indiscriminate use of antibiotics has led to the emergence of antimicrobial resistance in various isolates of bacteria (Ghosh *et al*., 2003). There is negligible data available on the hygienic aspects of meat including antibiogram status of microbes from local markets of Jammu.

Extensive review of literature reveals that not much work has been done in Jammu on the bacteriological quality of mutton or chicken meat. Keeping this in view, it is proposed to assess the hygienic quality of meat available in local markets of Jammu and assess contamination level in mutton and chicken with following objectives.

Objectives

1. To evaluate hygienic quality of raw mutton and chicken by total viable count.
2. Isolation and characterization of *E. coli, Staphylococcus aureus* and *Campylobacter* spp. in raw mutton and chicken.

CHAPTER- II

Review Of Literature

CHAPTER – II

REVIEW OF LITERATURE

Meat has been known for its nutritive composition which could explain why it is being consumed by many people worldwide. The protein profile of meat consists of amino acids that have been described as excellent due to the presence of all essential ones required by the body. A large proportion of the world's population rely on meat as source of food. During slaughtering and subsequent processes, microbial contamination of carcasses surface is unavoidable. Contaminants may cause infection to humans. Most of the microflora transferred to the carcasses during slaughtering processes may be non pathogenic but there is possibility that pathogens such as *Salmonella spp*. *Escherichia coli* O157:H7, *Campylobacter spp.* and *Listeria monocytogenes* may be present (Borch and Arinder, 2002). During recent years with the increase in global trade and awareness about the hygienic quality of the meat, international attention is being focused on ways to improve the microbial quality and safety. Microbes in meat, especially those causing food borne diseases in human beings, have recently become a matter of great public health concern. India could earn a considerable amount of foreign exchange through meat exports, but is confronted with major constraints such as poor quality and high level of microbial load (Huis In't Veld *et al.,* 1994).

2.1 Hygienic evaluation of raw meat

In recent years the microbial quality of meat as a food has become a great public health concern for the pathogenic bacteria of animal origin that result in food borne infections and intoxications. Poor hygiene and direct contact with infected materials during the production process leads to the contamination in meat. Higher demand for meat makes it mandatory to hygienically assess meat before it is declared fit for human consumption.

2.1.1 Hygienic evaluation of mutton

Mutton is a rich protein source is highly susceptible to contamination by microbes, which can lead to its breakdown, leading to food borne ailments in human, causes economic and health losses. Sheep may harbor pathogens even without displaying

any clinical signs. These pathogens may take refuge in the gastrointestinal tract or on exterior surfaces of sheep. Pathogenic food borne pathogens associated with mutton include *E. coli, Yersinia enterocolitica, Yersinia pseudotuberculosis, Salmonella* spp., *Campylobacter jejuni, Staphylococcus* spp. *Cryptosporidium parvum, Toxoplasma gondii.*

Krishnaswamy *and* Lahiry (1964) investigated mutton samples and reported the count to be 4.6 to 5.3 $\log_{10}$cfu/gm from market meat in India. Armitage (1995) on assessment of the microbiological quality of New Zealand beef and lamb reported that ±772 lamb carcasses had a mean Aerobic Viable Count (AVC) of 3.35cfu/cm^2. Gill and Baker (1998) assessed the hygienic performance of a sheep carcass dressing process in Canada and found unchilled sheep carcasses to have $\log_{10}$ AVC/cm^2 at the shoulder, loin and leg to be 2.81, 2.80 and 2.56, respectively. Bhandare *et al*., (2007) investigated the microbial load on sheep/goat carcasses in Deonar abattoir and traditional meat shops in Mumbai. The average total viable count after flaying, evisceration and washing in the abattoir was 5.51± 0.36, 6.06±0.53 and 5.13±0.58 cfu/cm^2, respectively. Pooled average TVC in the shops after flaying, evisceration and washing was 5.83±0.42, 6.48±0.27 and 6.17± 0.14 log cfu/cm^2, respectively.

Bhandare *et al.*, (2010) investigated chevon and mutton samples and found an average *Staphylococcus aureus* and *Staphylococcus epidermidis* counts as 3.15±0.18 and 3.46±0.17 $\log_{10}$cfu/cm^2, respectively. *Bacillus cereus, Bacillus subtilis* and *Clostridium* spp. counts were 3.10±0.08, 3.41 ±0.19 and 0.76±0.06 $\log_{10}$ cfu/cm^2 respectively. The *Escherichia coli* count was 3.54±0.06 and the *Klebsiella aerogenes* count was 3.22±0.22 $\log_{10}$ cfu/cm^2. Count for *Proteus vulgaris* and *Proteus mirabilis* were 3.44±0.14 $\log_{10}$ cfu/cm^2 and 3.71±0.1$\log_{10}$ cfu/cm^2 respectively highest prevalence was that of *S. epidermidis* followed by *K. aerogenes, B. subtilis* and *P. vulgaris* in from Mumbai.

Kumar *et al*., (2014) analysed samples of mutton and reported that total viable count to exceed the limit of 10,000cfu/g .The samples were positive for *Staphylococcus, Salmonella, E. coli, Listera* and *Salmonella*. Of the 50 meat samples analyzed, 9 (18%) samples of mutton exceeded viable count from Hyderabad region. Dabassa (2013) **reported lower aerobic counts (3.67log$_{10}$cfu/g) in mutton in Jimma. He reported that**

5.04percent of *E. coli* was isolated while 9 isolates were from beef, 8 from mutton and 5 from chevon and 4.2percent of *Klebsella* was isolated and 6 isolates were from beef, 3 from mutton and 1 from chevron, 1.26percent of *Salmonella* isolated , 3 isolates were from beef, 1 from mutton and 1 from chevron, 9.32percent of *Proteus* was isolated, 14 isolates were from beef, 8 from mutton and 11 from chevron, 9.57percent of *Staphylococcus* spp. was isolated, 19 isolates were from 9 beef and 10 each from mutton and chevon, respectively.

2.1.2 Hygienic evaluation of raw chicken meat

Poultry meat is more popular in the consumer market because of easy digestibility and acceptance by the majority of people (Yashoda *et al.,* 2001). The presence of pathogenic and spoilage microorganisms in poultry meat and its by-products remains a significant concern for suppliers, consumers and public health officials worldwide.

Johnston and Tompkin (1992) studied the microbiological quality in fresh chicken carcasses in the United States and reported total aerobic count to be 2 to 4 log cfu/cm^2. Mead *et al*., (1993) reported total aerobic count of 3.08-5.50 log10 cfu/g and coliforms count 2.2-3.80 log_{10} cfu/g and *Staphylococcus aureus* counts ranged between 2.3-3log_{10} cfu/g in fresh chicken meat processed under standard hygienic procedure in Britain. Abu-Ruwaida *et al.*, (1994) determined the microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait and reported the mean TVC, *E. coli* count and *S. aureus* count in chicken carcass as 6.5-6.6log_{10} cfu/g, 3.6 log_{10} cfu/g and 4.1log_{10} cfu/g respectively. Al-Mohizea *et al.,* (1994) reported that the mean initial microbial counts (log_{10} count/cm^2) in chicken carcass were 4.67, 4.14, 2.21, 2.78 and 2.96 for total aerobes, psychrotrophs, coliforms, *Staphylococcus aureus* and yeasts and moulds, respectively from Riyadh, Saudi Arabia. Sofos. J. N., (1994) determined the microbial load in poultry meat and reported that the total aerobic counts, *E. coli* count and *S. aureus* counts were 2-5 log_{10} cfu/g, 1-5 log_{10} cfu/g and 3log_{10} cfu/g respectively in London. Waldroup (1996) studied pathogens commonly associated with processed poultry meat and recorded that *S. aureus* counts in poultry meat varied more than 3 and less than 5 log_{10} cfu/g poultry meat and its by products from Nigeria. Pattanaik *et al.,* (1997) reported that the total viable count (TVC) and coliform count of the market

chicken samples were 7.93 and 7.22 $\log_{10}$ cfu/g, respectively in Bhubaneshwar city. Lillard (1989) studied the incidence and recovery of Salmonella and other bacteria from commercially grown poultry carcasses at selected pre and post evisceration steps and reported that total aerobic count of 3.71 $\log_{10}$ cfu/g. Bachhil from Izatnagar (1998) reported that on an average, 30percent each of fresh and frozen buffalo meats, 50percent kabab and 10percent curry samples were positive for *Staphylococcus aureus* with mean population of 1.00×10^4, 4.4×10^3, 1.09×10^3 and 2.10×10^2 per gram respectively. Out of 64 strains, 34.4 produced enterotoxin. Alvarez *et al.,* (2002) reported mean counts ($\log_{10}$ cfu/g) that ranged from 5.56 to 7.28, 5.96 to 7.87, 3.49 to 5.42, 2.60 to 4.33 and 2.47 to 3.48 for mesophiles, psychrotrophs, coliforms, *E. coli* and *S. aureus* respectively from retail chicken parts and processed chicken products in Spain. Khalifa and Abd El-Shaheed (2004) reported the aerobic plate count from raw chicken meat in Alexandria with an average of 3.0×10^4.*Enterobacteriaceae* were detected in all examined samples (100%) of raw chicken meat with mean values of 4.1×10^3 cfu/g. *Escherichia coli* occurrence was 22.6percent. *Staphylococcus aureus* was detected in 34.3percent of examined samples of raw chicken meat with an average counts of 2.8×10^2 cfu/g. Willayat *et al.,* (2006) in their study reported that 76(60.8%) of 125 fresh chicken sample in Srinagar city were contaminated with food borne micro-organisms and had mean viable counts of 3.74 cfu/g**.**

Abdellah *et al.,* (2007) reported levels of mesophiles, coliforms, *Escherichia coli* and *Staphylococcus aureus* on carcasses from 96 samples of chicken meat from popular market and artisanal (manually) slaughterhouses to be significantly higher ($P < 0.05$) than in poulterers' shops and supermarket in Morocco. Al Dughaym, A. M. (2009) analysed 100 samples of 10 poultry meat products in Saudi Arabia and reported the mean total bacterial counts to range from 2.7×10^4 cfu/g for nuggets to 3.3×10^7 cfu/g for burger and other products in the range of 10^5–10^6 cfu/g while *S. aureus* mean count ranged from $< 10^2$ cfu/g for all samples. Sengupta *et al*., (2011) reported that total aerobic bacterial count in chicken meat samples taken from Kolkatta procured from semi-urban and urban markets ranged from 51-55 x 10^4 and 4-25 x 10^4 cfu/g of chicken meat respectively. Mean coliform count per gram of poultry meat from semi-urban and urban markets were 3.20×10^2cfu/g and 6.50×10^2cfu/g for chicken meat, respectively.

Hygienic evaluation of raw meats

Dubal *et al.,* (2003) in Mumbai studied the Sheep/goat forequarters procured from freshly slaughtered animals decontaminated with hot water and inoculated with *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* Typhimurium. The Total viable count (TVC) of the treated meat samples was reduced by about 0.52 and 1.16 log units. Chandrashekhar *et al.,* (2010) reported that the $\log_{10}$ values of mean Total Viable Count was 6.14, 6.23, 6.12, 6.34 and mean coliform counts as 4.40, 3.75, 3.25, 3.38 cfu/g of meat, liver, heart and kidney from buffalo meat respectively at Mathura. Altug and Bayrak (2003) reported Standard Plate Counts that varied from 10^3 to 2.6×10^6 cfu/g, *Coliforms* varied from $<10^1$ to 2.4×10^4 cfu/g, *E. coli* varied from $<10^1$ to 3×10^2 cfu/g and *S. aureus* as 5×10^2 cfu/g from 68 Caviar samples in Iran. Shale *et al.,* (2005) studied *Staphylococcus* spp. on bovine meat in South Africa and the high *Staphylococcal* counts (1.7×106 cfu g^{-1}) were observed in the meat. Predominant species were *S. capitis, S. xylosus*, *S. auricularis*, *S. aureus* and *S. intermedius*. Crowley *et al.,* (2005) investigated the prevalence and numbers of *Enterobacteriaceae* in minced beef and beef burgers purchased from supermarkets and butchers shops in the republic of Ireland. Overall, in the 43 beef products in which *E. coli* 0157:H7 was present and the *Enterobacteriaceae* counts ranged from 0.52 to 6.98 $\log_{10}$ cfu/g. Biswas *et al.,* (2008) reported that buffalo meat trimmings (TT) samples had significantly higher ($P<0.001$) SPC, PTC, EFC, and SAC than silver sides(SS). *E. coli* was recovered from 32.4 percent of TT and 19.5percent SS samples from Izatnagar, Uttar Pradesh. Augustin and Minvielle (2008) reported in pork meat cuts from France that the contaminations were log normally distributed with *Enterobacteriaceae* mean log counts ranging from 0.6 to 2.2 $\log_{10}$ cfu cm^{-2} and *Pseudomonas* log counts ranging from 1.1 to 4.4 $\log_{10}$ cfu cm^{-2}.

Chaudhari *et al.,* (2008) analysed 50 beef samples from slaughter unit in Aizawland and revealed the average bacterial count of 6.13 ± 0.09 $\log_{10}$cfu/g and 12percent positive for *E. coli.* Yukshek *et al*., (2009) evaluated the microbial quality on ready to eat red meat donair and recorded $5.0\text{x } 10^6$, $3.1 \text{ x } 10^3$ and $2.1 \text{ x } 10^3$ for total aerobic mesophillic bacteria *E. coli*, *Enterococci* and coagulase positive *Staphylococci,* respectively and for chicken donair, kebabs they recorded $3.7\text{x}10^6$,$1.2 \text{ x}10^3$, $2.1\text{x}10^3$ and

$3.2x10^2$ total aerobic mesophillic bacteria, *E. coli. Enterococci* and coagulase positive *Staphylococci*, respectively from a local catering company in Bursa, Turkey. Lambey *et al.,* (2009) reported the log_{10} values of mean TVC for pigs and goats meat sample in Mathura as 7.78 cfu/g and 7.03 cfu/g respectively and mean coliform counts as 4.29 log_{10}cfu/g and 4.15 log_{10}cfu/g respectively. Feizullah and Daskalov (2010) reported the data showed that the total viable count of microorganisms (TVC) to vary between 4.09 and 6.79 log10 cfu/cm^2 on small ruminant carcasses (SR) slaughtered at the smaller facility, while in the larger factory, the values varied between 4.32 and 7.20 log10 cfu/cm^2 from Bulgaria. Bas *et al*., (2011) detected from beef carcasses the mean log_{10} APC/cm^2 to be 2.21 and *E. coli* was detected in 25 percent of carcasses (mean log positives -0.61/cm2). For sheep carcasses, corresponding values were 2.4 and log_{10} - 0.06/cm^2, respectively. For pig carcasses values were 2.81, 63percent and log_{10} - 0.23/cm^2, respectively. For skin off goat carcasses values were 1.15 percent, 27 percent and log_{10} - 0.38 /cm^2 , respectively from New South Wales, Austria.

Mawia *et al.,* (2012) found the mean values of log_{10} cfu/g of standard plate count (SAC), *E. coli* count (ECC), *S. aureus* count (SAC) and *Enterofaecal* count (EFC) of 85 chevon samples to be 6.37± 0.06, 3.85± 0.85, 3.98± 0.12 and 4.15± 0.15 and for poultry meat to be 6.65± 0.06, 3.81±0.11, 4.09±0.13, 4.02± 0.12 from Jammu. The overall prevalence of *E. coli* in chevon and poultry meat samples was 47(28.14%) out of 167 samples which include 22(25.88%) from chevon samples and 25(30.49%) from poultry meat samples. Singh *et al.,* (2014) assessed samples of poultry, carabeef, chevon and pork from Agra and reported SPC of poultry meat to be satisfactory but the level of contamination of samples of beef, chevon and pork were higher. For carabeef it was found to be 7.03±0.07, pork to be 6.86±0.02 and chevon to be 6.96±0.78.

2.2 Occurrence of bacterial pathogens in raw meat

Rao and Ramesh, (1988) reported predominant bacteria in fresh poultry meat from Mysore to be *Staphylococcus aureus, Micrococcus* and *Escherichia coli* and microbial spoilage of meat at higher temperatures was mainly due to mesophilic microorganisms. Mathieu *et al.,* (1991) reported that the majority (87.4%) of 190 *Staphylococcus aureus* isolates were from fresh beef in Lubumbashi (Zaire) that had

human origin *S. aureus* serovars. Vorster *et al.,* (1994) detected the incidence of *Staphylococcus aureus* and *Escherichia coli* in broilers meat in Pretoria, South Africa and found out that 23.4percent - 39.5percent of broiler meat was contaminated with *S. aureus* and 74.5percent of minced poultry meat by *E. coli*. Bachhil *et al.,* (1998) reported on an average, 30percent each of fresh and frozen buffalo meats, 50percent kabab and 10 percent curry samples to be positive for *Staphylococcus aureus* from Izzatnagar. Yadav *et al.,* (2001) reported that all the samples (100) were positive for coliforms, 49percent were positive for *E. coli* and 3.0percent were positive for *Salmonella* from sheep carcasses Mhow, Madhya Pradesh. Kumar *et al.,* (2001) isolated *Staphylococci* from 22 meat and meat products samples in Mumbai out of which 68.8percent were coagulase positive. Zhao et *al.,* (2001) found out that a total of 722 *Campylobacter* isolates from meat samples; 53.6percent of these isolates were *Campylobacter jejuni*, 41.3percent were *Campylobacter coli*, and 5.1percent were other species from chicken, turkey, beef and mutton in Greater Washington, DC.

Al-Gallas *et al.,* (2002) detected Shiga toxin-producing *E. coli* (STEC) strains. Among 250, *E. coli* strains isolated from 204 food samples (meat and dairy products) in Tunisia and found that Serotype O55:B5 to be the most prevalent type among *E. coli* isolates. Borch *et al.,* (2002) studied the bacteriological safety issues in red meat in Sweden and mention the importance of *E. coli* O157, *L. monocytogenes* and *S.* Typhimurium DT104 as meat-borne pathogens. Bailey *et al*., (2003) reported that the median prevalence and ranges for mutton sheep was to be 0-4percent and *Campylobacter* was found to be 73.7percent within (14/19) flock. Rathod *et al.,* (2004) reported that out of 60 chevon samples in Parbhani, the highest prevalence of 83.33percent and 100percent was recorded for Coliform and *Staphylococcus* respectively. Adwan *et al.,* (2004) isolated Shiga toxigenic *Escherichia coli* (STEC) from raw beef samples in Palestine and STEC was identified in 44 (14.7%) of 300 raw beef samples and 12 (27.3%) of the STEC isolates were serotype O157. Vazgecer *et al.,* (2004) investigated the microbiology of 72 chicken donar kebabs in Ankara, Turkey. The mesophillic aerobic counts ranges at 1.0x 10^2- 6.4x 105/g. *B. cereus, Staphylococci* and coliforms counts were less than 10^2 g for the total of 48percent, 50percent and 61percent of the samples, respectively. *E. coli* was found in 31percent of the samples ranged between 2.0x 10 and 5.0x 10^2/g. Stampi *et al.,*

(2004) detected *E. coli* in 45 (30.2%) of the 149 samples examined, mainly in the hamburger samples mixed with vegetables and in the loose minced beef. All the strains of *E. coli* O157 and most cases of *E. coli* were found in meat from small retailers in Italy. Hussein *et al.,* (2005) reported a wide ranges of prevalence rates of O157 (from 0.01% to 54.2%) and non-O157 (from 1.7% to 62.5%) STEC from beef carcass in Nevada, USA. Essid *et al.,* (2007) isolated *Staphylococcus xylosus* strains from a Tunisian traditional salted meat and found that all strains of *S. xylosus* had catalase activity and were able to reduce nitrates to nitrites. Hussain *et al*., (2007) processed 1636 food samples of meat, milk, and other food commodities from Pakistan and confirmed highest prevalence of *Campylobacter* in raw chicken meat (48%) followed by raw beef (10.9%) and raw mutton (5.1%).

Sharma and Singh., (2008) detected *E. coli* from meat samples taken from Himachal Pradesh. The prevalence of *E. coli* was highest in poultry meat (61.76 %), mutton (25.64 %) and chevon (22.09%). Singh *et al.,* (2009) collected a total of 86samples comprising of 35samples of chicken meat and 51 of carcasses swabs from local poultry farms and retail shops of Bareilly, Uttar Pradesh and were processed for detection and isolation of *C. jejuni* and *C. coli*. A total of 11isolates of *C. jejuni* and one of *C. coli* analysed 86 samples processed. Of the samples of chicken meat, 4 isolates of *C. jejuni* and one of *C. coli*, and from carcasses samples, 6 isolates of *C. jejuni* were recovered and the overall prevalence for *Campylobacter spp*. in poultry meat and carcasses was found to be 12.79percent. Lee *et al.*, (2009) reported that out of 3000 meat samples in Korea, 273 *E. coli* isolates were obtained from beef, poultry, and pork, resulting in an overall isolation rate of 9.1percent. Of these isolates, 201 were obtained from 1350 pork samples (14.9%), followed by 41 of 900 poultry samples (4.6%) and 31 of 750 beef samples (4.1%). Çadircia *et al.,* (2009) investigate the presence of *E. coli* O157 and O157:H7 strains from 200 ground beef and raw beef samples in Turkey. *E. coli* O157 was detected in five of the 200 (2.5%) samples tested, whereas *E. coli* O157:H7 was not detected in any sample. Boston *et al.,* (2009) investigated the prevalence of thermophillic *Campylobacter spp*. (TCS) in 198 beef and 120mutton carcass excision samples, and 232 chicken carcasses samples randomly collected from different retail stores and meat processing plants in Istanbul. TCS were isolated from 11.1percent,

21.6percent and 50.4percent of beef, mutton and chicken samples tested, respectively. A total of 292 *Campylobacter* isolates were obtained from the samples (56.5%) *C. jejuni*, (33.9%) *C. coli* and (9.6%) *C. lari*. *C. jejuni* was the species most commonly isolated from chicken meat (56.5%) while *Campylobacter coli* was the most common in beef (63.3%) and mutton (63.9%) carcasses. There was no significant seasonal variation in the prevalence of TCS.

Nastasijevic *et al.,* (2009) detected occurrence of *E. coli* O157, 0percent, 6.2 percent and 2.1 percent, respectively, in 106 samples of beef trimmings, 48 samples of minced beef and 48 samples of batter intended for production of raw, fermented sausages in Serbia. Abd Abbas (2010) who detected 40percent *S. aureus* from raw mutton samples from Bagdhad abattoir. Rahimi *et al*., (2010) determined prevalence and antimicrobial resistance of *Campylobacter spp*. isolated from chicken carcasses during different stages of broiler processing in a major commercial poultry processing plant in Southwestern Iran. Overall, 84 chicken carcasses were sampled from 4 sites along the processing line during a total of 7 visits. In addition, 14 water samples from the chiller tank were also analysed. Using the cultural method, 186 of 336 (55.4%) carcasses were positive for *Campylobacter*. *C. jejuni* being more frequently isolated (89.4%) than *C. coli* (10.6%). Ahmad *et al.,* (2013) found that *E. coli* positive samples to be significantly higher for beef outlets as compared to beef abattoirs (75% vs 40%), sheep outlets as compared to sheep abattoirs (55% vs 30%), and goat outlets as compared to goat abattoirs (50% vs 20%). The 45percent of the chicken samples collected from retail outlets were also positive for *E .coli* with mean *E. coli* counts of 2.74 $\log_{10}$cfu/cm^2 in Lahore, Pakistan. Rahimi *et al.,* (2014) reported that 249 samples tested were positive for *Enterobacteriaceae*. The level of contamination with *Enterobacteriaceae* in raw meats ranged from 3.26 $\log_{10}$ cfu/g to 4.94 $\log_{10}$ in Tennessee. Ashraf *et al*., (2015) reported that out of 280 different meat samples, contamination rate was 54.58percent for *Salmonella enteritidis*, (53.75%) *E. coli*, (27.08%) for *S. aureus* and 17.8% *Bacillus* in Pakistan.

Studies on antibiotic sensitivity test for *E. coli, S. aureus* and *Campylobacter spp.*

Multidrug resistance of bacteria isolates is an emerging public health problem as multidrug resistant bacteria are showing an alarming increase during recent years and is now fast emerging public health problem. The main risk factor for an increase in bacterial resistance is an increased use of antibiotics for therapy and prevention of bacterial infections as well as growth promoter.

Antibiogram pattern of *E. coli*

Saha *et al*., (2003) studied the occurrence of *E. coli* from broiler birds in Bengal and their antibiogram. The highest sensivity was recorded against cefotaxime (79.17%) followed by norfloxacin (77.08%), enrofloxacin (73.96%) and amikacin (67.71%). Lowest sensivity was recorded against ampicillin (2.08%), penicillin G (3.13%), cephalexin (13.54%), erythromycin (13.54%) and nalidixic acid (15.63%). Yadav and Sharda (2006) studied the drug resistance of *Escherichia coli* isolated from mutton and revealed highest sensitivity to chloramphenicol (95.92%) followed by colistin (89.80%), ceftriazone (75.51%), amikacin (69.39%), ciprofloxacin (67.35%), gentamicin(67.35%), tetracycline (59.18%), nalidixic acid (48.98%), cotrimoxazole (46.94%) and ampicillin (8.16%). Aksoy *et al*., (2007) observed the verotoxin production in strains of *Escherichia coli* isolated from cattle and sheep, and their resistance to antibiotics. The antibiotic resistance rates of *E. coli* strains reported as follows: tetracycline (51.6%), streptomycin (24.2%), ampicillin (13.1%), amoxicillin/clavulanic acid (5.2%), gentamycin (4.6%), ciprofloxacin (4.6%), trimethoprim-sulfamethoxazole (4.3%), ceftaxime (0.7%). None of the strains were found resistant to cefepime or ceftazidime. Hossnera *et al.,* (2007) observed 100percent resistance for nalidixic acid and ampicillin whereas high sensitivity for ciprofloxacin, erythromycin and cloxacillin of *E. coli* isolates from broiler meat in Bangladesh.

Yadav *et al*., (2007) reported that among the 15 isolates of *E. coli* tested for resistance against various antibiotics all the isolates (100%) were found to be resistant to erythromycin and streptomycin, followed by sulphadiazine (95.84%) and cephaloridine (87.50%). Moderately high resistance was detected towards cephalexin (41.69%),

penicillin G (37.60%), ceftiofur (33.36%) and norfloxacin (33.36%), enrofloxacin (27.40%) and carbenicillin (25.30%). Multiple drug resistance was demonstrated in ten isolates of *E. coli* in 100 mutton sample showing simultaneous resistance to 2 to 10 antibacterial agents in Mhow, India. Dhanushree and Mallya (2008) observed that 77.5% of the *E. coli* isolates were resistance for ampicillin whereas 80percent, 90percent and 82percent isolates shows sensivity for cephoaxime, ciprofloxacin and ceftriazone respectively from the meat samples in Mangalore.

Antibiogram pattern of *S. aureus*

Lukasova and Jarchovska (1979) studied resistance to selected antibiotics in 325 strains of *Staphylococcus aureus*, isolated from foodstuffs. Out of these strains, 50.46percent were resistant to penicillin, 15.7%percent ampicillin, 4percent to streptomycin, 18.2percent to oxytetracyclin, 2.5percent to gentamicin, 1.6percent to kanamycin, 53.5percent to colistin and 9.2percent to bacitracin. Eighty five strains (26.15%) were sensitive to all the antibiotics used; 107 strains (32.92%) were resistant to one antibiotic, and 133 strains (40.93%) to two or more antibiotics from Czecho slovakia. Uzeh *et al.,* (2006) studied the bacterial contamination of raw meat and Tshire Suya, a Nigerian meat product and found out that *S. aureus* shows high sensivity against ciprofloxacin, ofloxacin and erythromycin. Pereira *et al.,* (2007) studied the antibiotic susceptibility of *S. aureus* isolates from various foods in Portugal and observed that 70percent and 73percent of *S. aureus* strains were resistant to ampicillin and penicillin, respectively. No resistance to nitrofuantoin, vancomycin and ciprofloxacin was found. A small percentage of the isolates demonstrated resistance to rifampicin, gentamicin, gentamicin, erythromycin, chloramphenicol and tetracycline.

Khatoon *et al.,* (2010) isolated 115 *S. aureus* from laboratories situated in different areas of Karachi out of which 85percent were resistant to ampicillin, 43percent against kanamycin, 23percent against gentamycin, 5percent against chloramphenicol and 40percent against methicillin. Only 8percent were resistant to ciprofloxacin and vancomycin. Waters *et al*., (2011) characterized the prevalence, antibiotic susceptibility profiles, and genotypes of *S. aureus* from meat and poultry samples. Resistance (intermediate and complete) to tetracycline, ampicillin, penicillin, and erythromycin was

highly prevalent but resistance to other antimicrobial was also observed, including dalfopristine, fluoroquinolones, oxacillin , daptomycin and vancomycin from the United States of America.

Datta *et al.,* (2012) isolated *S. aureus*. From seventy nine samples, percentage resistance of the *S. aureus* samples to penicillin, ampicillin, streptomycin, tetracycline, amoxicillin and neomycin were found to be 85.71percent, 71.42 percent, 100 percent, 71.42 percent, 100 percent and 85.71 percent. The percentage of multidrug resistant (MDR, resistant against more than three antibiotics) *S. aureus* was 20percent respectively in Dhaka, Bangladesh. Karmi (2013) observed that 44percent (11/25), 51percent (13/25), 40percent(10/25), 24percent (6/25) and 44percent (11/25) of isolates of *S. aureus* were positive for methicillin-resistance tests for freshly slaughtered whole chicken carcasses, chicken portions, chicken luncheon, chicken sausages and chicken burgers respectively. Higher contamination rate of MRSA (methicillin resistant *Staphylococcus aureus*) was found in raw poultry meat and the lower rate in poultry meat products subjected to heat treatment and preservatives in Egypt.

Antibiogram pattern of *Campylobacter*

Khurana and Kumar (1996) found that all 24 *Campylobacter jejuni* and 7 *Campylobacter coli* isolates were sensitive to gentamicin and streptomycin and resistant to penicillin, ampicillin and cloxacillin from 105 poultry meat samples from Bareilly. Smith *et al.,* (1999) isolated ciprofloxacin resistant *Campylobacter* from 20percent of the retail chicken products. Resistant *C. jejuni* being 14percent and resistant *C. coli* from 5percent samples. Ciprofloxacin resistant *C. jejuni* were also found resistant to grepafloxacin, trovafloxacin and sarafloxacin and most were resistant to levofloxacin. Varma *et al.* (2000) suggested that nalidixic acid, gentamicin and enrofloxacin can be used for the effective control of Campylobacter infection as all the 27 (100%) isolates of *C. jejuni* were sensitive to three antibiotics.

Kolar *et al*., (2002) found out 11 *Enterococcus* sp. strains to be resistant to vancomycin (vancomycin-resistant enterococci – VRE) out of 228 strains in poultry chicken in Czech Republic, Europe. Pezzotti *et al*., (2003) studied the resistance of *C.*

jejuni and *C. coli* to various antibiotics and also compared to human isolates. The workers reported that *C. coli* were more resistant than *C. jejuni* and the resistance to quinolones was frequently observed in *C. coli* isolates of chicken meat. All *C. coli* isolates from different sources like chicken meat, broilers, beef, pork and humans were resistant to tetracyclins from Italy. Rahimi *et al.* (2010) determined the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from chicken carcasses during different stages of broiler processing in a major commercial poultry processing plant in Southwestern Iran. Overall, 84 chicken carcasses were sampled from 4 sites along the processing line during a total of 7 visits. In addition, 14 water samples from the chiller tank were also analyzed. Using the cultural method, 186 of 336 (55.4%) carcasses were positive for *Campylobacter*. *C. jejuni* being more frequently isolated (89.4%) than *C. coli* (10.6%).

Pallavi and Kumar (2014) reported that all the isolates of *Campylobacter* spp. (150) chicken meat samples to be resistant to co-trimoxazole but sensitive to erythromycin. Awoubo *et al*., (2010) detected high resistance rates of *Campylobacter* isolates to cephalothin (84%), cephalexin (61%), ampicillin (58%), streptomycin (43%) and cotrimoxazole (43%). Upadhyay *et al.*, (2016) reported that all of the *C. jejuni* and *C. coli* isolates were resistant to cephalothin (100%) and sensitive to gentamicin and erythromycin (100%). While 80percent, 50percent and 40percent resistance was observed against suphamethoxazole, ampicillin and ciprofloxacin respectively. Among *C. coli*, 70, 80 and 50 percent sensitivity was observed against ciprofloxacin, nalidixic acid and gentamicin respectively and 50percent isolates were resistant to ampicillin in Pantnagar.

Chapter- III

Material and Methods

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MATERIALS AND METHODS

3.1 PLACE OF WORK

The study was conducted at Division of Veterinary Public Health and Epidemiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-J, R.S Pura, Jammu and Kashmir, India. The period of study was from May, 2015 to April, 2016.

3.2 CHEMICALS AND REAGENTS

Chemicals and reagents used in the study were procured from Hi-Media (India), and other reputed national manufacturers. Glassware and other materials of routine use were cleaned and sterilized following the standard procedure. Media and antibiotics used in the present study were procured from Hi-Media. Chemicals and reagents used were from reputed national and international firms. Details of media /chemicals used in the present study are listed below:

NSS (Normal Saline Solution)

Ingredients	**Amount (gm/ litre)**
Sodium Chloride	800 mg
Distilled Water	100 ml

Dissolved 800 mg of sodium chloride solution in 100 ml of distilled water and sterilized by autoclaving at 15 lbs (121^0C) for 15 minutes

Plate Count Agar (M 091 Hi Media, Mumbai)

Ingredient	**Amount (gm/ litre)**
Casein enzyme hydrolysate	5
Yeast extract	2.5
Dextrose	1.0
Agar	15.0

Suspended 23.5 grams in 1000 ml of distilled water. Boiled to dissolve the medium completely. Sterilized by autoclaving at 15 lb (121°C) for 15 minutes.

MacConkey's Agar (M 081 Hi Media, Mumbai)

Ingredient	**Amount (gm/litre)**
Peptic digest of animal tissue	20
Bile salts	10
Sodium chloride	5
Neutral red	0.07
Agar	15
Final pH (at 25^0c)	7.4±0.2

55.07 grams were added in 1000 ml of distilled water. Heated to boiling to completely dissolve the medium. Sterilized by autoclaving at 15 lb (121°C) for 15 minutes.

Mannitol Salt Agar (MH118-500G, Hi-Media, Mumbai):

Ingredient	**Amount (gm/litre)**
Peptone	10
Meat extract	1
D-Mannitol	10
Sodium chloride	75
Phenol Red	0.025
Agar	20
Distilled water	1000 ml
Final pH	7.1 ± 0.2

111.02 gm of media was suspended in 1000 ml distilled water and heated to dissolve. It was then sterilized in an autoclave at 121° C for 15 mins

EMB Agar (M317-500G, Hi-Media, Mumbai)

Ingredients	**Amount (gm/litre)**
Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	5.000
Sucrose	5.000
Eosin – Y	0.400
Methylene blue	0.065
Agar	13.500
Final pH (at 25°C)	7.2±0.2

35.96 gm of media was suspended in 1000 ml distilled water and mixed well until suspension was uniform. The medium was heated to dissolve completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes (overheating was avoided). The medium was cooled to 45-50°C and was shaken to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculent precipitate.

Muller Hinton Agar (M 173 Hi Media, Mumbai)

Ingredient	**Amount (gm/litre)**
Beef infusion	300
Casein acid hydrolysate	17.50
Starch	1.5
Agar	17.0

38.00 grams were added in 1000 ml of distilled water. Heated to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 lb (121°C) for 15 minutes.

Nutrient Agar (M001-500G Hi Media, Mumbai)

Ingredients	**Amount (gms/litre)**
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15
Distilled water	1000 ml

Final pH (at 25^{o}c) 7.3±1

Dissolved 37.0 grams in 1000 ml in distilled water and sterilized by autoclaving at 15 lbs pressure (121^{0}C) for 15 minutes.

Simmon's Citrate Agar (M099 Hi-Media, Mumbai)

Ingredients	**Amount (gm/litre)**
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0
Final pH (at 25°C)	6.8±0

Brain Heart Infusion Broth (M2101 Hi-Media, Mumbai)

Ingredients	**Amount (gm/litre)**
Peptic digest of animal tissues	10.0
Calf brains, infusion (solids)	12.5
Beef heart infusion (solids)	5.0
Sodium chloride	5.0
Dextrose	2.0
Disodium phosphate	2.5
Final pH (At 25°C)	7.4

37 gm of dehydrated media was suspended in 1000 ml distilled water, distributed in test tube and sterilized by autoclaving at 15 psi pressure, 121°C temperature for 20 minutes.

Nitrate Agar (M072-500G, Hi-Media, Mumbai)

Standard formula	**Amount (gm/litre)**
Peptic digest	5.0
Beef extract	3.0
Potassium Nitrate	1.0
Agar	12
Final pH (at 25^0C)	6.8± 0.2

Dissolved 21.0 grams in 1000ml in distilled water. Heat to boiling to dissolved the medium completely. Dispensed in tubes and sterilised by autoclaving at 15 lbs pressure (121^oC) for 15 minutes. Allowed the tubes to solidify slanted position.

Peptone Water (RM001-500G, Hi-Media, Mumbai)

Ingredient	Amount (gm/litre)
Peptone	10 g
Sodium chloride	5 g
Distilled water	1000 ml
Final pH	7.2± 0.2

Suspended 15 grams in 1000 ml distilled water and heated to dissolve the medium completely. Dispensed in tubes and sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes.

DNase Test Agar base (M482-100G, Hi-Media, Mumbai)

Ingredient	Amount (gm/litre)
Casein enzymic hydrolysate	15.00
Papaic digest of soyabean meal	5.00
Deoxyribonucleic acid (DNA)	22.00
Sodium chloride	5.00
Agar	15.00
Final pH (at 25°C)	7.3 ± 0.2.

42.0 gm of agar was suspended in 1000 ml distilled water and heated with frequent stirring for proper mixing and sterilized at 121°C for 15 mins at 15 lbs psi followed by pouring in plates.

Preparation of Nitrate broth

Ingredient	Grams/litre
Tryptose	20
Disodium phosphate	2
Agar	1
Potassium Nitrate	1
pH	7.2±0.2

25 grams of medium was dissolved in 1000 ml distilled water, dispensed in tubes and autoclaved at 121^0C and 15 Ibs pressure for 15 minutes.

Urea Agar Base (Christensen's) (Hi Media-M112S)

Ingredient	**Grams/litre**
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Disodium phosphate	1.2
Monopotassium Phosphate	0.8
Phenol red	0.8
Agar	15.0
pH	6.8±0.2

24 grams of urea agar base was dissolved in 950 ml distilled water, autoclaved at 121^0C and 15 Ibs pressure for 15 minutes and cooled at 45^0C. Then 50 ml of sterile 40% urea solution was added, mixed well and dispensed in tubes and cooled in slanted position.

Preston Broth Base (M899, Hi-Media, Mumbai)

Ingredient	**Grams/litre**
Peptic digest of animal tissue	10.0g
Beef extract	10.g
Sodium chloride	5.0g
Final pH (at 25^0C)	7.5±0.2

25 grams of broth base dissolved in 1000 ml distilled water.

Brucella Agar Base (Hi- Media-M074) dehydrated

Ingredient	**Grams/litre**
Peptone	5g
Beef extract	5g
Dextrose	10g
Agar	15g
NaCl	5g

21.5 grams of Brucella agar base was rehydrated in 500ml of distilled water, heated to boiling to completely dissolve and autoclaved at 15 lbs. pressure for 15 minutes, cooled to 45^0C and 5-7% defibrinated, sheep blood was added, followed by addition of Butzlers antibiotic supplement and *Campylobacter* growth supplement mixed gently and finally poured into the plates and were kept at 37^0C over night and checked for any growth.

Butzlers Antibiotic Supplement: (FD007, HiMedia, Mumbai)

Ingredient	**Grams/litre**
Bacitracin	12500 units
Actidione (cyclohexamide)	25 mg
Colistin sulphate	5000 units
Novobiocin	2.5 mg
Cephazolin sodium	7.5 mg

Vial content dissolved in 2.5 ml of distilled water sufficient for 500 ml media without frothing.

CHEMICALS/KITS

Kovac's reagent	R008-100 ml, Hi-Media, Mumbai.
Gram's Staining Kit	K001, Hi-Media, Mumbai
Methyl Red	1007, Hi-Media, Mumbai
Oxidase Discs	DD018, Hi-Media, Mumbai
α- napthol	R009-100ML, Hi-media, Mumbai
Kovac's reagent	R008-100ml, Hi-media, Mumbai
Ninhydrin	GRM248-25G, Hi-Media,Mumbai
Sodium Hippurate	RM6523-100, Hi-Media,Mumbai

3.3 COLLECTION OF SAMPLES

A total number of 145 samples (25gm), 75 from mutton and 70 from chicken meat were collected in sterilized packs and were transported in ice box with all aseptic precautions. The samples were randomnly collected from local markets of Jammu described in Table 3.1. After collection, all the samples were labelled accordingly and held at 4^0C until examination. The time between sample collection and analysis was analysed within 2-3hrs after collection.

Table 3.1: **Area wise sample collection from different parts of Jammu city**

S.No.	Area	Mutton	Chicken
1	Sunjwan	15	20
2	Kacchi-Chhawni	15	10
3	Bathindi	15	5
4	Janipur	10	10
5	Gujjar Nagar	10	10
6	Narwal	5	10
7	Ambphala	5	5
	Total	**75**	**70**

3.4 Enumeration of Standard plate count (SPC), *E. coli* count (ECC) and *Staphylococcus aureus count* (SAC)

SPC, ECC, and SAC in the samples were enumerated following the methods of American Public Health Association (APHA, 1984) with suitable modifications whenever necessary. For serial dilution, a 25 g portion of meat sample was aseptically weighed and triturated to 225 ml of sterile normal saline solution (NSS) so as to give 10^{-1} dilution. The sample was homogenized for uniform dispersion. This was further serially diluted 10 fold till 10^{-5} dilution. The number of CFU per gram of test sample (N) was calculated using the formula adopted from Diane *et al.*, (1995).

$$N=C/V\ (n_1+0.1n_2)\ d$$

Where C = sum of the colonies on all plates counted

V = volume applied to each plate

n_1 = no. of plates counted at first dilution

n_2 = no. of plates counted at second dilution

d = dilution from which first count was obtained

3.4.1 Standard Plate Count (SPC)

For evaluating Standard Plate Count (SPC), the spread plate technique was followed using 10^{-4} and 10^{-5}dilutions. Briefly, 0.1 ml of the two dilutions were spread plated in duplicate on solidified plates of plate count agar and incubated at 37 ± 2^0C for 24 hrs. The plates containing between 30-300 colonies at two consecutive dilutions were selected to calculate the results.

3.4.2 *E. coli* count (ECC)

E. coli were isolated and enumerated using MacConkey agar (Hi Media, Mumbai, India). Briefly, 0.1 ml of 10^{-2} and 10^{-3} dilution were spread plated in duplicate on dried plates of MacConkey agar and incubated at 37˚C for 24 hrs. The presumptive colonies were determined by counting number of sharp pinkish colonies with about 0.5 mm diameter. The colonies were confirmed by streaking 2-3 colonies on to EMB agar (Eosin-

methylene blue agar) and colonies with typical metallic sheen were further confirmed by Gram's staining and by biochemical tests. The average numbers of colonies were recorded as $\log_{10}$cfu/g of sample.

3.4.3 *S. aureus* count (SAC)

Baired Parker agar was used for isolation and enumeration of *Staphylococcus aureus*. Breifly, 0.1ml of 10^{-2} and 10^{-3} dilution were spread plated in duplicate on dried plates of Baired parker agar with egg yolk tellurite emulsion (Hi-Media Mumbai, India) and incubated at 37^0C for 24 hrs. The presumptive colonies were determined by typical morphology (grey-black, shiny convex colony with a narrow entire margin surrounded by a zone of clearing) and by streaking 2-3 colonies on MSA (Mannitol salt agar). Colonies were further confirmed by Gram staining and biochemical tests. Average numbers of colonies were recorded as log cfu/g of sample.

3.2 INTERPRETATION OF RESULTS

Guideline levels for determining the microbiological quality for raw meat (Bureau of Indian Standard, 1995; FSSAI, 2011)

	Satisfactory	**Marginal**	**Unsatisfactory**
Standard plate count (cfu/g)	$<10^4$	$<10^5$	$\geq 10^5$
E. coli (cfu/g)	*<3*	3-100	*≥100*
S. aureus (cfu/g)	$<10^2$	10^2-10^3	10^3–10^4

3.5 ISOLATION AND IDENTIFICATION OF BACTERIAL PATHOGENS

The isolation and identification of *E. coli*, *S. aureus* and *Campylobacter* was achieved by using selective media for each bacterium followed by Gram staining of presumptive colonies and Standard biochemical testing (Cowan, 1974; Cruikshank *et al*., 1975).

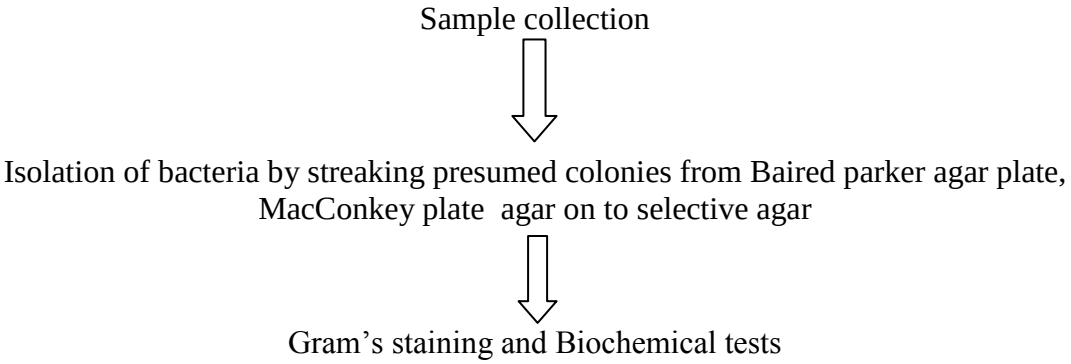

Figure 1: Flow diagram of procedures for isolation of *Campylobacter*

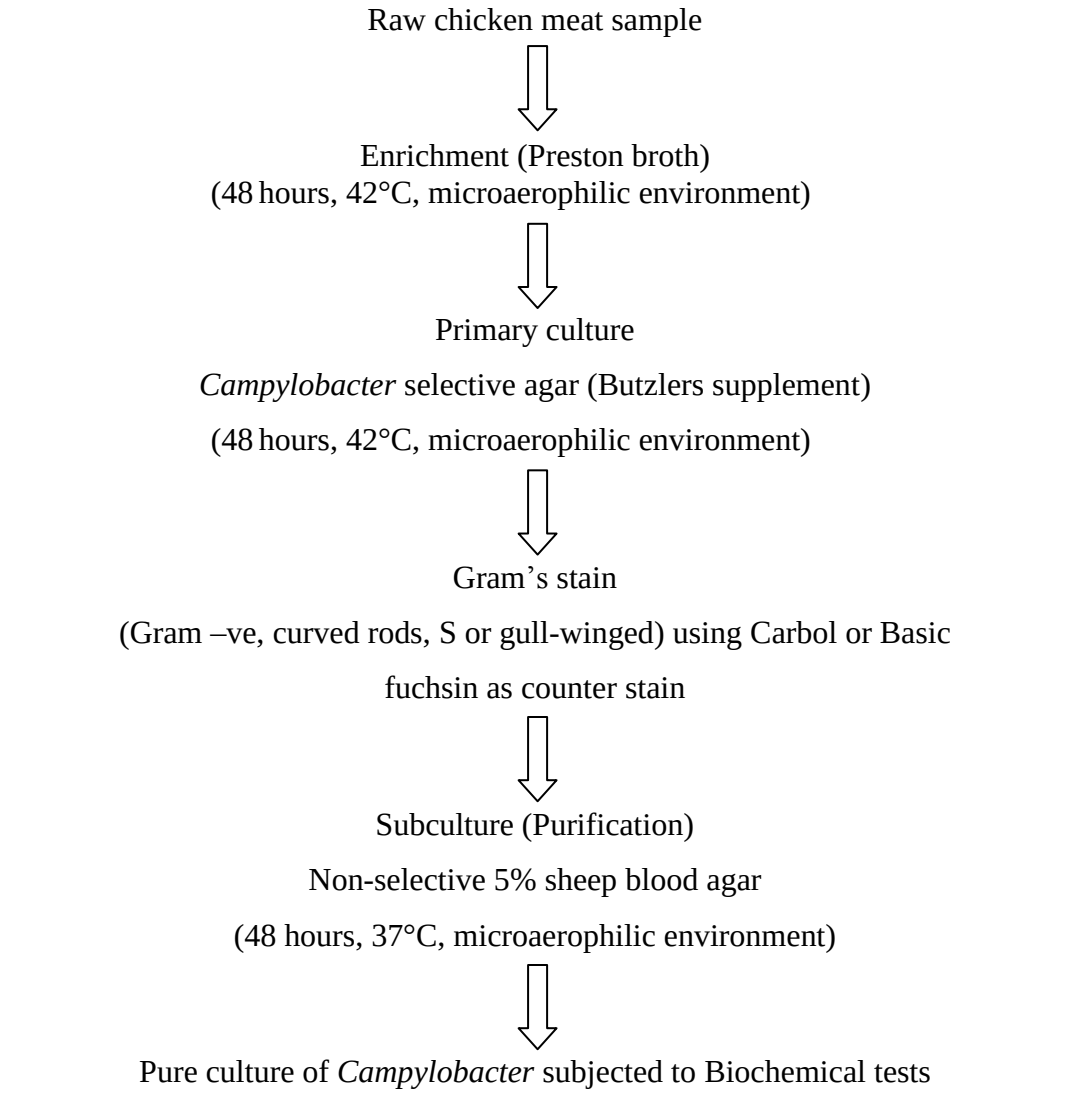

Figure 2: Flow diagram of procedure for isolation of *Campylobacter* spp.

3.5.1 Isolation of *E. coli*

From MacConkey plate pink colour colonies were presumed to be *E. coli*. Three to four assumed colonies were then streaked on EMB agar with incubation for 24 hr at 37^0C. A green metallic sheen on EMB agar indicates the presence of *E. coli.* Gram staining revealed Gram negative rods. On biochemical testing, *E. coli* isolates were found to be catalase positive, oxidase negative, indole positive, methyl red positive, Voges Proskauer negative, citrate negative, gas production positive on TSI agar.

3.5.2 Isolation of *S. aureus*

From Baird-Parker agar, 3-4 presumed colonies with characteristics (circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet black, frequently surrounded by a halo) suggestive for *S. aureus* were selected and streaked on mannitol salt agar. Isolates having yellow colour colonies on mannitol salt agar after incubation at 37^0C were presumed to be *S. aureus*. The presumed colonies were analysed for Gram staining. The colonies having Gram positive cocci arranged like bunches of grapes were purified on brain heart infusion agar and subjected to biochemical tests *viz.,* catalase, oxidase, coagulase, DNase test, IMViC and hemolysis (Bennett and Lancette, 2001) for confirmation.

3.5.3 Isolation of *Campylobacter*

Twenty five grams of poultry meat samples were cut into small pieces and homogenized in 75 ml of Normal Saline Solution (NSS) 10 ml of homogenate was transferred to Preston enrichment broth and incubated at 42^0C for 48 hrs under microaerophilic conditions.

Samples from broth were streaked onto Brucella Agar Base and incubated at 42°C for 48hrs under microaerophilic environment These plates were kept in candle extinction jar to produce microaerophilic conditions along with a nutrient agar plate heavily inoculated with *E .coli* (Saha and Sanyal, 1989). Plates were checked daily for 5 days for the growth of typical colonies exhibiting watery or dew drop appearance and the suspected isolates were further confirmed by Grams staining and standard biochemical methods for further testing.

3.5.5 Identification and characterization of isolated bacterial pathogens

3.5.5.1 Gram's staining

All the presumed cultures of *Staphylococcus* spp., *E. coli* and *Campylobacter* were subjected to Gram's staining and observed for Gram's reaction, size, shape and arrangement of cells.

Table 3.3: Characteristics of bacterial pathogens on selective agar and Gram's staining

S.No.	Bacteria	Media used	Colony characteristics	Gram's Staining
1	*E. coli*	i) MacConkey agar ii) Eosin methylene blue agar	i) Pink to rose-red colonies. Colonies may be surrounded by a zone of precipitated bile ii) Green metallic sheen on EMB	Gram negative rod shaped bacterium
2	*S. aureus*	i) Baird- Parker agar ii) Mannitol salt agar	i) Circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet black colonies, frequently surrounded by a halo on BPA ii) Yellow coloured colonies on MSA	Gram positive cocci (grape like clusters)
3	*Campylobacter*	Brucella agar base	Watery and dew drop like swarming type colonies	Gram negative, Comma, S-shaped rods, helical, Seagull appearance

Table 3.4: Characteristics of *E. coli* and *S. aureus* in various biochemical tests

S.No.	Biochemical test	Positive/Negative	
		E. coli	***S. aureus***
1	Catalase test	+	+
2	Oxidase test	-	-
3	Indole test	+	-
4	Methyl red test	+	+
5	Voges-Proskauer test	-	+
6	Citrate utilization test	-	+
7	Nitrate reduction test	+	+
9	Triple Sugar iron	Gas production	-

Table 3.5: Typical biochemical reactions of commonly isolated species of thermophilic *Campylobacters* (Goossens and Butzler, 1992).

Characteristics	*C. jejuni*	*C. coli*
Catalase	+	+
Oxidase	+	+
Nalidixic acid	S	S
Cephalothin	R	R
Nitrate reduction	+	+
Indoxyl acetate hydrolysis	+	+
Hippurate hydrolysis	+	-
Urease test	+	+
H_2S production	+	_

3.5.5.2 Biochemical Characterization

3.5.5.2.1 Catalase test

A colony of bacteria was taken over a clean microscopic slide, a loopful of 3 per cent H_2O_2 was added. Prompt effervescence indicated catalase production indicating a positive result.

3.5.5.2.2 Oxidase test

In order to carry out this test, oxidase discs were used. Colony of bacterium was rubbed over the oxidase discs. No change in colour indicated negative reaction while blue colouration was a positive result.

3.5.5.2.3 Indole test

This test demonstrates the ability to decompose the amino-acid tryptophan to indole. The test culture was inoculated in 2 % tryptone water followed by incubation at 37^0C for 48 to 96 hours. After incubation, 0.5 ml Kovac's reagent was added to the culture medium and shaken gently. Appearance of red colour indicated positive reaction.

3.5.5.2.4 Methyl red test

The test was performed by inoculating glucose phosphate peptone water (MR-VP broth) with test organism and incubated at 37^0C for 24 to 48 hrs. Appearance of red colour upon addition of methyl red indicator indicated positive reaction.

3.5.5.2.5 Voges- Proskauer test

Five ml of glucose phosphate peptone water (MR-VP Broth) Hi-Media Pvt. Ltd. was inoculated with isolated organism and incubated at 37^0C for 48 hours. Then 1 ml of 40 per cent potassium hydroxide and 3 ml of 5 per cent α-napthol (in absolute ethanol) were added. Appearance of red colour indicated a positive reaction.

3.5.5.2.6 Citrate utilization test

Simmons's citrate medium was used to detect ability of the organism to utilize citrate as the sole source of carbon and energy for growth and an ammonium salt as the sole source of nitrogen. The test was carried out by inoculating Simmon's citrate media with test organism and incubating at 37^0C up to 48-96 hrs. Change in green solid slant to blue indicated a positive reaction. No change in colour indicated negative results.

3.5.5.2.7 Triple sugar iron test (TSI)

The test organism was inoculated on TSI agar (Hi-Media, Mumbai, India). The needle was stabbed to the bottom of the butt and then the needle was drown over the slant so as to produce sufficient surface growth and incubated at 37^0C for 24hrs. After incubation, the TSI agar was examined carefully to estimate different reaction. The tubes showed acid butt (yellow), acid slant with gas production and no H_2S production for *Escherichia coli.*

3.5.5.2.8 Nitrate Reduction Test

This test is based on the ability of certain organism to reduce nitrate in media to nitrite which is detected by the addition of alpha napthylamine and sulphanilic acid and forms a pink red compound. This test was carried out by inoculating nitrate media with the test organism and incubated at 37^0C upto 24hrs. Appearance of red colour with drop of sulphanilic acid and alpha naphthylamine reagent indicate a positive reaction.

3.5.5.2.10 DNase test for detection of Thermonuclease

DNA hydrolysis test or Deoxyribonuclease (DNase) test is used to determine the ability of an organism to hydrolyze DNA to utilize it as a source of carbon and energy for growth. The test is used to differentiate *S. aureus* from other Staphylococci, which do not produce the enzyme responsible for hydrolysis. The DNase test is particularly useful when plasma is not available to perform the "Coagulase test" or the results of a coagulase test are difficult to interpret as there is high correlation between *S. aureus* for coagulase and DNase production. For this test, Staphylococcus isolate was streaked on DNase agar plate and incubated at 37^0C for 24 hrs. After that 1percent HCl was poured over it. The

plates with positive results showed a clear zone around the streaked area indicating DNA hydrolysis.

3.5.5.2.11 Coagulase test

Coagulase converts fibrinogen to fibrin by activating a coagulasee reacting factor present in plasma detected by clotting in the test tube which convert fibrinogen directly by clumping of *Staphylococcal* cells. In tube test method, 0.5 ml of human plasma and 0.5 ml of 18-24 hrs pure broth (BHI) culture of the *S. aureus* was added is sterile test tube aseptically. It was then mixed by gentle rotating of the tube. The tube was incubated at 37^0C in water bath for 4 hrs and observed for the formation of visible clot after every 30minutes. The reaction is positive if any degree of clotting is visible within the tube.

3.5.5.2.12 Urease Test

A loopful of test culture was inoculated into Urease agar slants. The slants were observed every 6 hrs for change of color upto 2 days. The positive reaction was indicated by change of color from yellow to pink, red, while no color change was considered as negative.

3.5.6 Specie characterization for *Campylobacter* spp.

Biochemical characterization of the isolates which were presumptively identified as genus *Campylobacter* was done for species identification as follows:

3.5.6.1 Hippurate Hydrolysis test

A loopful of *Campylobacter* culture was inoculated into 1 ml of sodium hippurate solution. The inoculated tubes were incubated at 42^0C for 4 hrs under microaerophilic conditions. Then 0.5 ml of ninhydrin solution was added and reincubated at 42^0C for 10 minutes. A positive reaction was indicated by appearance of deep blue color.

3.5.6.2 Sensitivity to Cephalothin (30 µg) and Nalidixic acid (30 µg).

To perform the drug sensitivity test disc diffusion method with Muller-Hinton agar supplemented with 5% sheep blood was used. 48 hour old cultures under test were inoculated with sterile swab on the plates. After drying the plates, sensitivity discs of Nalidixic acid (30 g) and Cephalothin (30 g) were placed on the plates. Plates were

incubated at 42^0C for 48 hrs under microaerophilic conditions in candle extinction jar. A zone of inhibition of atleast 3mm around a disc indicated that the strain under test is sensitive to that antibiotic used.

3.5.6.3 H_2S production in Triple Sugar Iron agar

A loopful of culture was inoculated into butt and slant and incubated at 37^0C for 24 hrs. H_2S production was indicated by blackening of the media.

3.5.6.4 Indoxyl acetate hydrolysis

Loopful growth of colony was placed on indoxyl acetate soaked discs and 1 drop of sterilized distilled water was added. It was incubated for 5-10 min at 37^0C. Development of dark blue color was considered as positive test.

3.6 ANTIBIOTIC SENSIVITY TEST OF THE ISOLATED PATHOGENS

3.6.1 Antimicrobial drug sensitivity and resistance pattern of *E. coli* and *S. aureus* isolates

All confirmed isolates (*E. coli* and *S. aureus*) were examined for their antimicrobial drug susceptibility/resistance pattern by disc diffusion technique of Bauer *et al*., (1966). Ten antimicrobial discs used were obtained from HiMedia Laboratories Pvt. Ltd. Mumbai. Interpretation of the isolates as sensitive and resistant was determined as per supplier's instructions. Inoculum for culture sensitivity test was prepared by inoculating colonies of isolates in 5ml Mueller Hinton Broth and incubated at 37^0C for 24 hrs till light to moderate turbidity develops. Plates of Mueller Hinton Agar were seeded with about 100 µl of inoculums using sterile cotton swabs. The inoculated plates were allowed to dry. Antibiotic discs were placed on inoculated agar surface about 2 cm. from one another. The plates were incubated at 37^0C for 16-18hrs and diameter of the zones of inhibition were measured using HiMedia scale.

3.6.2 Antimicrobial drug sensitivity and resistance pattern of *Campylobacter* isolates.

Antimicrobial susceptibility of *Campylobacter* spp. was performed by the disc diffusion method of Bauer *et al.*, (1966). A loopful of growth from Butzlers selective media was taken and mixed with 0.5 of normal saline to make a fine suspension. A sterile cotton swab was dipped in bacterial suspension to be tested. The cotton swab was rubbed gently over the plate in several directions by rotating the plate to obtain uniform distribution of inoculums. After drying the plates, discs were placed manually using a sterile fine forcep. The seeded plates were incubated at 37^0C in microaerophilic atmosphere. The results were taken after 24 hr as per direction of the manufacturer. The list of antibiotics with concentration used in this study are shown in Table 3.6

Table 3.6: List of Antibiotics used for study of sensitivity and resistance pattern of isolated pathogens.

S.No.	Antibiotics discs used for isolated pathogens		
	E. coli	***S. aureus***	***Campylobacter spp***
1	Ciprafloxacin(5mcg)	Ciprafloxacin(5mcg)	Ampicillin (10mcg)
2	Co-trimoxazole(25mcg)	Co-trimoxazole(25mcg)	Cotrimoxazole(25mcg
3	Ampicillin(100mcg)	Ampicillin(100mcg)	Norfloxacin(15mcg)
4	Amikacin(30mcg)	Amikacin(30mcg)	Ciprafloxacin(5mcg)
5	Chloramphenicol(10mcg)	Chloramphenicol(10mcg)	Chloramphenicol(30mcg))
6	Tetracyclin(30mcg)	Tetracyclin(30mcg)	Tetracyclin(30mcg
7	Polymyxin B(300units)	Polymyxin B(300units)	Erythromycin (15mcg)
8	Amoxycillin(10mcg)	Amoxycillin(10mcg)	Cephalothin(30mcg)
9	Nalidixic acid(30mcg)	Nalidixic acid(30mcg)	Nalidixic acid (30mcg)
10	Gentamicin(100mcg)	Gentamicin(100mcg)	Gentamicin(10mcg)

Chapter- IV

Results

CHAPTER – IV

RESULTS

In the present study a total of 145 samples comprising of raw mutton (n = 75) and chicken (n = 70) from different areas of Jammu city were analysed to evaluate hygienic status by quantifying bacteria. The quantification was done by standard plate count (SPC), *E. coli* count (ECC) and *Staphylococcus aureus* count (SAC) methods. The isolates were identified on the basis of cultural, morphological and biochemical characters and their antibiotic sensitivity pattern was also studied. The results of the same are presented as below.

4.1 ISOLATION AND IDENTIFICATION OF BACTERIAL PATHOGENS FROM RAW MEAT (MUTTON AND CHICKEN)

On analysis of 145 samples of raw meat (mutton and chicken), a total of 122 isolates (52 *E. coli*, 49 *S. aureus* and 21 *Campylobacter*) were obtained. The preliminary isolation of *E. coli* and *S. aureus* was done by streaking presumed colonies of bacteria from *E. coli* count plates and *S. aureus* count plates to selective media and for *Campylobacter*, it was done using enrichment protocol followed by isolation on selective agar; the identification and confirmation of isolates was done on the basis of Modified Gram's staining, growth on other selective agars and biochemical tests. None of the mutton sample yielded *Campylobacter*.

4.1.1 Identification and confirmation of *E. coli*

E. coli isolates comprised the highest number (n= 52) being 35.9percent of the bacterial isolates of which 23(30.6%) isolates from raw mutton and 29(41.4%) isolates were from raw chicken meat samples. In the present study, *E. coli* isolates exhibited pink to rose-red colonies on MacConkey agar (plate 3) and EMB agar, these colonies produced green metallic sheen (plate 4). Gram staining revealed Gram negative rods (plate 8). On biochemical characterization, they were oxidase negative, catalase positive, indole positive (plate 11), methyl red positive (plate no. 12), Voges Proskauer negative

(plate 13), citrate negative (plate 14), TSI (plate 15) it showed acid with gas production and nitrate positive (plate 16). Overall 52 isolates of *E. coli* were obtained.

4.1.2 Identification and confirmation of *S. aureus*

A total of 49 (33.7%) isolates of *S. aureus* were obtained comprising of 24 (32%) isolates from raw mutton and 25 (35.7%) isolates from raw chicken. In the present study, *S. aureus* isolates exhibited jet black colonies surrounded by white halo (lecithinase activity) on Baird Parker agar indicating presumptive *S. aureus* (plate 5). When further streaked on MSA, typical pale yellow, opaque colonies of *S. aureus* were produced (plate 6). Gram's staining revealed Gram positive cocci grape like cluster (plate 9). On biochemical characterization, they revealed oxidase negative, catalase positive (plate 17), indole negative (plate 18), methyl red positive (plate 19), Voges Proskauer positive (plate 20), citrate positive (plate 21) and coagulase positive (plate 22) and DNase positive (plate 23). Thus, 49 isolates of *S. aureus* were obtained.

4.1.3 Identification and confirmation of *Campylobacter*

Twenty-one (14.5%) isolates of *Campylobacter* were obtained from 145 raw meat samples however, none of isolate of *Campylobacter* could be isolated from mutton. *Campylobacter* showed dew drop colony on selective medium (plate 7). On Gram staining it showed Gram negative Comma shaped, helical, seagull shaped rods (plate 10) and on biochemical characterization it showed catalase positive (plate 24), oxidase positive (plate 25), urease positive (plate 29) nitrate positive (plate 27), and Out of 21 isolates of *Campylobacter* spp., 16 (76.1%) *C. jejuni and* 5 (23.8%) *C. coli* were characterized by indoxyl acetate test (plate 26), hippurate hydrolysis test (plate 28) and H_2S production on TSI test (plate 30) with no gas production.

4.2 Occurrence of pathogens in raw mutton

Out of 75 mutton samples, the highest occurence of *S. aureus* was found in raw mutton samples to be 24(32%) while for *E. coli*, the occurence was comparatively lower 23(30%) and no sample of mutton was positive for *Campylobacter*. Table 4.1 shows occurence of bacterial pathogens in different areas of Jammu from raw mutton.

Table 4.1: Area wise Occurrence of bacterial pathogens in raw mutton samples (n=75)

S.No.	Area	*E.coli*	*S. aureus*	*Campylobacter*
1	Sunjwan (n=15)	6(40%)	6(40%)	0
2	Kacchi Chhawni(n=15)	4(26.6%)	4(26.6%)	0
3	Bathindi (n=15)	3(20%)	4(26.6%)	0
4	Janipur (n=10)	4(40%)	2(20%)	0
5	Gujjar Nagar (n=10)	3(30%)	4(40%)	0
6	Narwal (n=5)	3(60%)	1(20%)	0
7	Ambphala (n=5)	0(0%)	3(60%)	0
8	**Total (n=75)**	**23(30.6%)**	**24(32%)**	**0**

4.3 Occurrence of bacterial pathogens in raw chicken meat samples

Out of 70 raw chicken meat samples, the highest occurrence of *E. coli* was found to be 29(41.4%) while for *S. aureus*, the occurrence was slightly lower 25(35.5%). Twenty one (30%) of *Campylobacter* were obtained from 70 raw chicken meat samples. The results are presented in Table 4.2 shows occurrence of bacterial pathogens collected from raw chicken in different areas of Jammu.

Table 4.2: Area wise occurrence of bacterial pathogens in raw chicken samples (n=70)

S.No.	Area	*E. coli*	*S. aureus*	*Campylobacter*
1	Sunjwan (n=20)	9(45%)	6(40%)	6(30%)
2	Kacchi Chhawni (n=10)	5(50%)	4(26.6%)	4(40%)
3	Bathindi (n=5)	3(20%)	4(26.6%)	1(20%)
4	Janipur (n=10)	5(50%)	2(20%)	3(30%)
5	Gujjar Nagar (n=10)	4(20%)	4(40%)	4(40%)
6	Narwal (n=10)	2(20%)	1(20%)	3(30%)
7	Ambphala (n=5)	1(20%)	3(60%)	0
	Total(n=70)	**29(41.4%)**	**25(35.7%)**	**21(30.0%)**

4.4 Occurrence of *Campylobacter spp*. in raw chicken meat samples

Out of 70samples of raw chicken meat, 21isolates of *Campylobacter* were obtained. From 21 isolate of *Campylobacter, C. jejuni* isolates were found to be more in numbers 16(76.1%) compared to *C. coli* which were found to be less 5(23.8%). The results are presented in table 4.3.

Table 4.3: Occurrence of *Campylobacter* spp. in chicken meat samples.

Type of sample	*Campylobacter* spp. isolated		Percentage occurrence
	C. jejuni	***C .coli***	21(30.0%)
Poultry meat (70)	16(76.1%)	5(23.8%)	

4.5 EVALUATION OF HYGIENIC QUALITY OF RAW MEAT

4.5.1 Standard plate count (SPC) of raw mutton samples

The results of SPC revealed the mean values (log_{10}cfu/g) of raw mutton samples to be 6.12±0.08. The highest SPC count was found from Narwal area with mean value 6.43±0.05 and the lowest count was found to be 5.89 ±0.12 in Kachhi Chhawni. The lowest range was found to be 5.3 from Sunjwan and Amphbala while highest range 6.6 was found in samples from Janipur area from Jammu city (Table 4.4a).

Table 4.4(a): SPC (log_{10} cfu/g) in mutton (n=75)

Area	Range	SPC (log_{10} cfu/g)
Sunjwan (n=15)	5.3-6.5	6.19 ±0.19
Kacchi Chhawni (n=15)	5.4-6.4	5.89 ±0.12
Bathindi (n=15)	6.3-6.5	6.36 ±0.08
Janipur (n=10)	5.5-6.6	6.00± 0.17
Gujjar Nagar (n=10)	5.4-6.5	6.12± 0.15
Narwal (n=5)	6.2-6.5	6.43 ±0.05
Ambphala (n=5)	5.3-6.5	5.91 ±0.26
Total (n=75)		**6.12±0.08**

4.4.2 *E. coli* count (ECC) of raw mutton samples

After confirmation of positive samples of *E. coli*, the results of ECC revealed the mean values (log_{10}cfu/g) of mutton samples to be 3.30±55. The highest ECC count was found in samples from Gujjar Nagar area with mean value of 3.96±0.35 and the lowest count was found to be 3.70±0.13. The lowest range was found to be 3.2 from Sunjwan area while highest range was found to be 4.4 in samples from Kacchi Chhawni area from Jammu city (Table 4.4b). The raw mutton samples (n=5) from Ambphala area did not reveal the presence of *E. coli.* The results are presented in table 4.4(b).

Table 4.4(b): ECC (log_{10}cfu/g) of in mutton (n=75)

Area	Samples positive for ECC	ECC(log_{10} cfu/g)	
		Range	Mean
Sunjwan (n=15)	6(60%)	3.2-4.0	3.69± 0.13
Kacchi Chhawni (n=15)	4(26.6%)	3.3-4.4	3.90± 0.02
Bathindi (n=15)	3(20%)	3.9-4.2	4.04 ± 0.11
Janipur (n=10)	4(40%)	3.2-4.1	3.75± 0.22
Gujjar Nagar (n=10)	3(30%)	3.5-4.2	3.96± 0.35
Narwal (n=5)	3(60%)	3.5-4.2	3.70± 0.13
Ambphala (n=5)	0(0%)	ND	ND
Total (n=75)	23(30.6%)		3.30± 0.55

ECC are given as log_{10}cfu /g of positive samples only
ND= Not detected

4.4.3 *Staphylococcus aureus* (SAC) (log_{10}cfu /g) of raw mutton

After confirmation of positive samples of *S. aureus,* the results of SAC revealed the mean values (log_{10}cfu/g) of mutton samples to be 4.08±0.15. The highest SAC count was found from Sunjwan area with mean value to be 4.46 ± 0.03and the lowest count was found to be 3.28 ±0.00 from Narwal. The highest range was found to be 4.6 from Sunjwan while lowest was found to be 3.1 from Kacchi Chawni area from Jammu city (Table 4.4c).

Table4.4(c): SAC(log_{10} cfu/g) in mutton (*n*=75)

Area	Samples positive for SAC	SAC (Log_{10}cfu/g)	
		Range	Mean
Sunjwan (n=15)	6(40%)	4.2-4.6	4.46 ± 0.03
Kacchi Chhawni (n=15)	4(26.6%)	3.1-4.4	3.99± 0.00
Bathindi (n=15)	4(26.6%)	3.9-4.4	4.33± 0.00
Janipur (n=10)	2(20%)	3.6-4.2	3.95± 0.01
Gujjar Nagar (n=10)	4(40%)	4.1-4.5	4.32 ± 0.07
Narwal (n=5)	1 (20%)	3.28	3.28 ±0.00
Ambphala (n=5)	3(60%)	3.5-4.4	4.28± 0.00
Total (n=75)	**24(32%)**		**4.08± 0.15**

4.5.1 SPC of raw chicken meat samples

The results of SPC revealed the mean values (log_{10}cfu/g) of 70 raw chicken samples to be 6.17 ±0.05. The highest SPC count obtained in sample from Sunjwan area with mean value 6.33± 0.16 and the lowest count was found to be 5.94± 0.22 from Kacchi Chhawni. The lowest range was found to be 5.2 from Sunjwan area while highest range was found to be 6.7 from Narwal area from Jammu city (Table 4.5a).

Table 4.5(a): SPC (log_{10} cfu/g) of raw chicken meat samples (n=70)

Area	Range	SPC (log_{10} cfu/g)
Sunjwan (n=20)	5.2-6.5	6.33± 0.16
Kacchi Chhawni (n=10)	4.9-6.2	5.94± 0.22
Bathindi (n=5)	6.1-6.4	6.16 ±6.17
Janipur (n=10)	6.2-6.6	6.32 ±0.17
Gujjar Nagar (n=10)	5.9-6.4	6.18± 0.20
Narwal (n=10)	6.1-6.7	6.22± 0.22
Ambphala (n=5)	5.5-6.4	6.08 ±0.27
Total (n=70)		**6.17 ±0.05**

4.5.2 ECC of raw chicken samples

After confirmation of positive samples of *E. coli*, the results of ECC revealed the mean values (log_{10}cfu/g) of chicken samples to be 3.99±0.13. The highest ECC count was found from Sunjwan with mean value to be 4.22 ±0.07 and the lowest count was found to be 3.23 ±0.00 from Ambphala. The lowest range was found to be 2.9 in samples from Kacchi Chhawni while highest range 4.7 was in samples from Gujjar Nagar area in Jammu city (Table 4.5b).

Table 4.5 (b): ECC (log_{10} cfu/g) of chicken samples (n=70)

Area	Samples positive for ECC	ECC(log_{10} cfu/g)	
		Range	Mean
Sunjwan (n=20)	9(45%)	3.5-4.4	4.22 ±0.07
Kacchi Chhawni (n=10)	5(50%)	2.9-4.2	3.99± 0.00
Bathindi (n=5)	3(20%)	3.6-4.4	4.08± 0.05
Janipur (n=10)	5(50%)	3.1-4.2	3.97± 0.00
Gujjar Nagar (n=10)	4(40%)	4.1-4.7	4.27 ±0.06
Narwal (n=10)	2(20%)	3.5-4.3	4.17± 0.04
Amphala (n=5)	1(20%)	-	3.23 ±0.00
Total (n=70)	**29(41.4%)**		**3.99 ±0.13**

4.5.3 SAC (log_{10} cfu/g) of raw chicken samples

After confirmation of positive samples of *S. aureus*, the results of SAC revealed the mean values (log_{10}cfu/g) of mutton samples to be 4.16 ±0.09. The highest SAC count was found in samples from Kacchi Chhawni with mean value to be 4.42±0.04 and the lowest count was found in samples to be 3.87 ±0.44 from Gujjar Nagar. The highest range was found to be 4.8 from Kacchi Chhawni while lowest range was found to be 3.6 from Sunjwan area from Jammu city (Table 4.5c).

Table 4.5 (c): SAC (log_{10} cfu/g) of chicken samples (n=70)

Area	Samples positive for SAC	SAC(log_{10} cfu/g)	
		Range	Mean
Sunjwan (n=20)	7(35%)	3.6-4.2	4.11± 0.06
Kacchi Chhawni (n=10)	4(40%)	4.3_4.8	4.42 ±0.04
Bathindi(n=5)	3(60%)	3.6-4.1	3.94± 0.00
Janipur (n=10)	6(60%)	4.3-4.7	4.46 ±0.04
Gujjar Nagar (n=10)	4(40%)	3.3-4.1	3.87 ±0.44
Narwal (n=10)	4(40%)	3.9-4.7	4.39 ±0.11
Ambphala (n=5)	1(20%)	3.99	3.99 ±0.00
Total (n=70)	25(35.7%)		**4.16 ±0.09**

4.6 ANTIBIOGRAM PATTERN STUDIES

4.6.1 Antibiogram pattern of *E. coli*

E. coli isolates were found to be most sensitive to ciprofloxacin (88.46%) followed by polymyxin B (78.84%), co-trimoxazole (76.92%), chloramphenicol (76.92%). High resistance was found to be against ampicillin (84.61%) followed by nalidixic acid (73.07%). Low resistance was found to be against ciprofloxacin (5.76%) and co-trimoxazole (5.76%).

Table 4.6: Antibiotic sensitivity and resistance pattern of *E. coli* isolates (n=52)

S.No.	Antibiotics	No. of isolates of *E. coli*		
		Sensitive	**Intermediate**	**Resistant**
1	Ciprafloxacin (5mcg)	46(88.46%)	3(5.76%)	3(5.76%)
2	Co-trimoxazole (25mcg)	40(76.92%)	9(17.30%)	3(5.76%)
3	Ampicillin (100mcg)	5 (9.61%)	3(5.76%)	44(84.61%)
4	Amikacin (30mcg)	2(3.81%)	23(44.23%)	27(51.42%)
5	Chloramphenicol (10mcg)	40(76.92%)	6(11.53%)	6(11.53%)
6	Tetracycline (30mcg)	13(25%)	3(5.76%)	36(69.23%)
7	Polymyxin B (300units)	41(78.84%)	4(7.69%)	7(13.46%)
8	Amoxycillin (10mcg)	39(75%)	9(17.60%)	4(7.69%)
9	Nalidixic acid (30mcg)	11(21.15%)	3(5.76%)	38(73.07%)
10	Gentamicin (100mcg)	2(3.84%)	31(59.61%)	19(36.53%)

4.6.2 Antibiogram pattern *of S. aureus*

S. aureus was found to be most sensitive to ciprofloxacin (91.83%) followed by amikacin (91.83%), polymyxin B (83.67%) and chloramphenicol (73.46%). Low resistance was found to be against ciprofloxacin (0%) followed by amikacin (0%) and polymyxin B (8.16%).Higher resistance was found to be against nalidixic acid (100%) followed by tetracyclin (61.22%).

Table 4.7: Antibiogram pattern of *S. aureus* (n=49)

S.No.	Antibiotics	No. of isolates of *S. aureus*		
		Sensitive	Intermediate	Resistant
1	Ciprafloxacin (5mcg)	45(91.83%)	4(8.16%)	0(0%)
2	Co-trimoxazole (25mcg)	20(40.81%)	23(46.93%)	6(12.24%)
3	Ampicillin (100mcg)	13(26.53%)	16(32.65%)	20(40.81%)
4	Amikacin (30mcg)	45(91.83%)	4(8.16%)	0(0%)
5	Chloramphenicol (10mcg)	36(73.46%)	8(16.32%)	5(10.20%)
6	Tetracycline (30mcg)	13(26.53%)	6(12.24%)	30(61.22%)
7	Polymyxin B (300units)	41(83.67%)	4(8.16%)	4((8.16%)
8	Amoxycillin (10mcg)	25(51.02%)	6(12.24%)	18(36.73%)
9	Nalidixic acid (30mcg)	0(0%)	0(0%)	49(100%)
10	Gentamicin (100mcg)	16(32.65%)	25(51.02%)	8(16.32%)

4.6.3 Antibiogram pattern of *Campylobacter jejuni* isolates (n=16)

Campylobacter jejuni was found to be 100% sensitive to nalidixic acid followed by erythromycin (93.75%), chloramphenicol (87.5%) and norfloxacin (87.5%). *C. jejuni* isolates were found to be (100%) resistance to cephalothin followed by ampicillin (81.25%)

Table 4.8: Antibiogram pattern of *Campylobacter jejuni* (n=16)

S.no.	Antibiotics	No. of isolates of *C. jejuni* in chicken		
		Sensitive	Intermediate	Resistant
1	Ampicillin (10mcg)	1(6.25%)	2(12.5%)	13(81.25%)
2	Gentamicin (10mcg)	14 (87.5%)	2(12.5%)	0(0%)
3	Nalidixic acid (30mcg)	16(100%)	0(0%)	0(0%)
4	Ciprafloxacin (5mcg)	9(56.25%)	5(31.25%)	2(12.5%)
5	Tetracycline (30mcg)	13(81.25%)	2(12.5%)	1(6.25%)
6	Cephalothin (30mcg)	0(0%)	0(0%)	16(100%)
7	Erythromycin (15mcg)	15(93.75%)	1(6.25%)	0(0%)
8	Chloramphenicol (30mcg)	14(87.5%)	2(12.5%)	0(0%)
9	Cotrimoxazole (25mcg)	3(18.75%)	2(12.5%)	11(68.75%)
10	Norfloxacin (15mcg)	14(87.5%)	0(0%)	2(12.5%)

4.9 Antibiogram pattern of *Camylobacter coli* isolates (n=5)

C. coli isolates were found to be most sensitive to nalidixic acid (100%) followed by ciprofloxacin (80%), erythromycin (80%), chloramphenicol (80%). *C. coli* isolates were found to be (100%) resistant to cephalothin followed by ampicillin (80%).

Table 4.9: Antibiogram pattern of *Campylobacter coli* (n=5)

S.No.	Antibiotics	No. of isolates of *C. coli* in chicken		
		Sensitive	Intermediate	Resistant
1	Ampicillin (10mcg)	0(0%)	1(20%)	4(80%)
2	Gentamicin (10mcg)	3 (60%)	2(40%)	0(0%)
3	Nalidixic acid (30mcg)	5(100%)	0(0%)	0(0%)
4	Ciprafloxacin (5mcg)	4(80%)	1(20%)	0(0%)
5	Tetracycline (30mcg)	1(20%)	2(20%)	3(60%)
6	Cephalothin (30mcg)	0(0%)	0(0%)	5(100%)
7	Erythromycin (15mcg)	4(80%)	1(20%)	0(0%)
8	Chloramphenicol (30mcg)	4(80%)	1(20%)	0(0%)
9	Cotrimoxazole (25mcg)	1(20%)	1(20%)	3(60%)
10	Norfloxacin (15mcg)	3(60%)	0(0%)	2(40%)

S. aureus and E. coli were found to be most sensitive to ciprofloxacin and higher resistance was found to be against nalidixic acid. *Campylobacter* spp. isolates were found to be most sensitive to nalidixic acid and most resistant to cephalothin.

Plate 1: Standard plate count on plate count agar medium

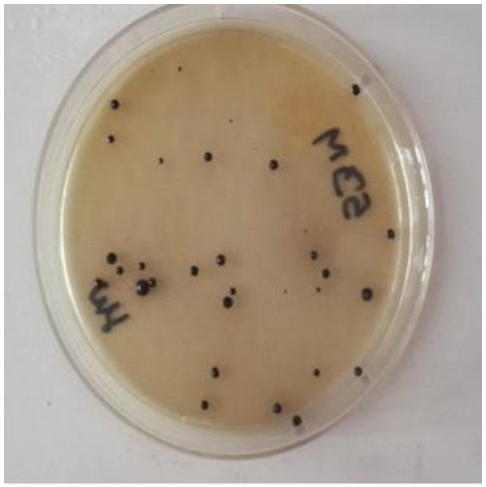

Plate 2: S. aureus count on Baired Parker agar medium

Plate 3: Pink colonies of *E. coli* on MacConkey agar plate.

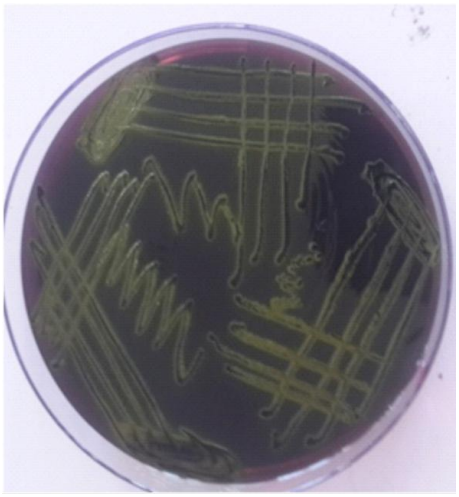

Plate 4: Greenish metallic sheen of *E. coli* on EMB

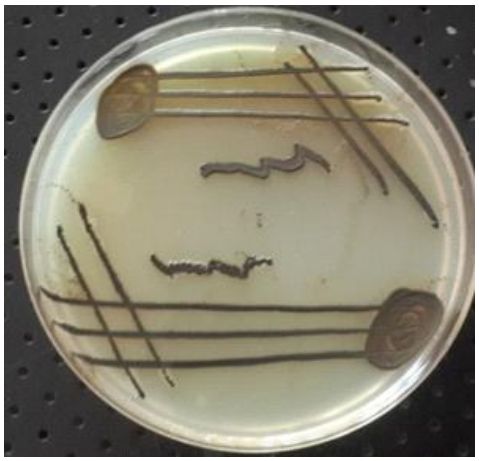

Plate 5: Jet black colonies surrounded by white halo (lecithinase activity) on Baird Parker agar indicating presumptive *S. aureus*

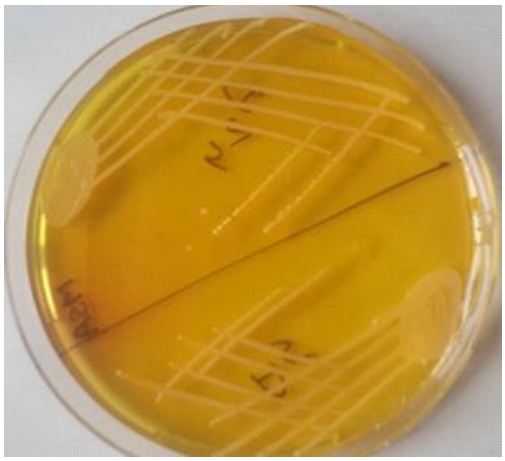

Plate 6: Typical pale yellow, opaque colonies of S.aureus on Mannitol Salt agar.

Plate 7 : Dew drop colonies of *Campylobacter* on Brucella agar base

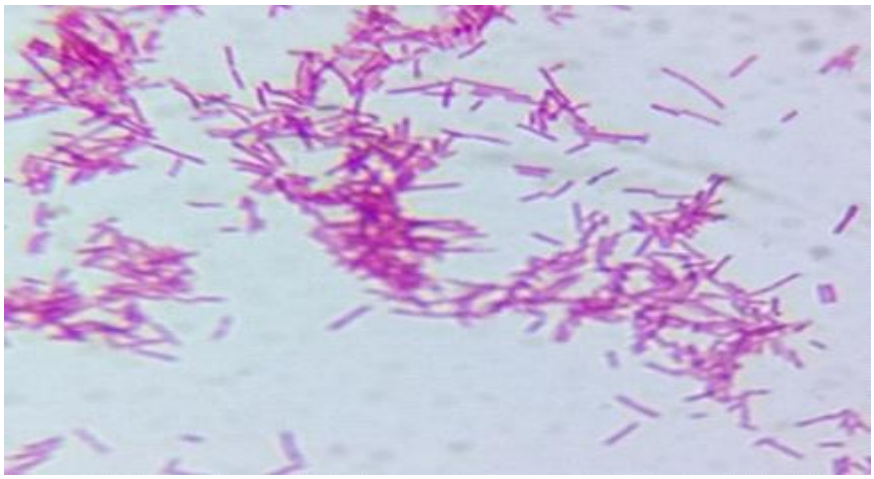

Plate 8 : Gram's staining showing rod shaped *E. coli*

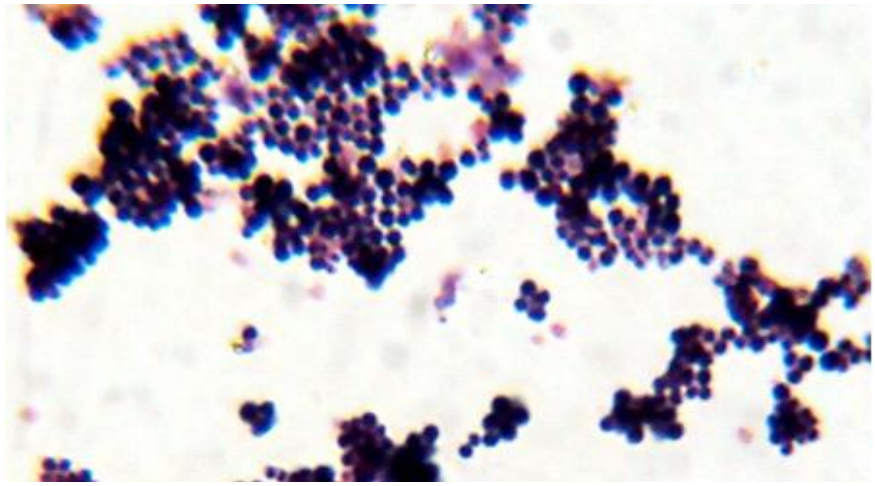

Plate 9 : Gram's staining showing grape bunch shaped *S. aureus*

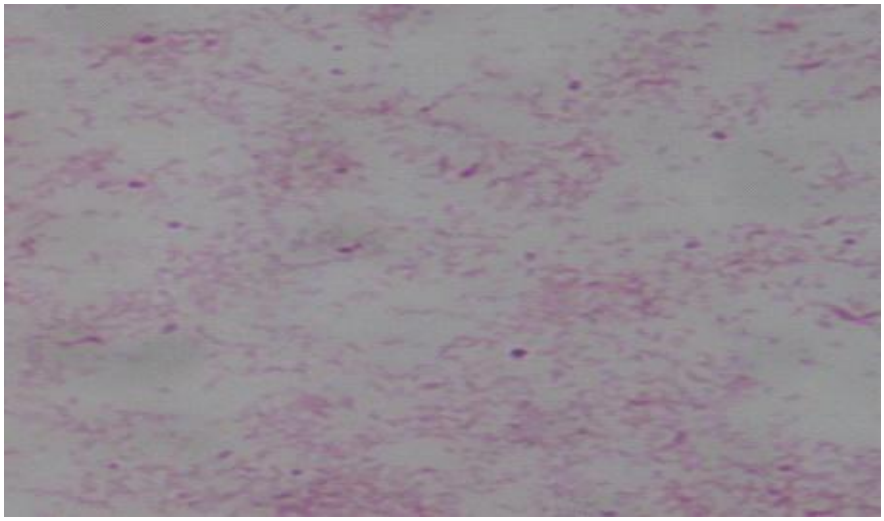

Plate 10 : Gram's staining showing comma shaped, seagull appearance of *Campylobacter spp.*

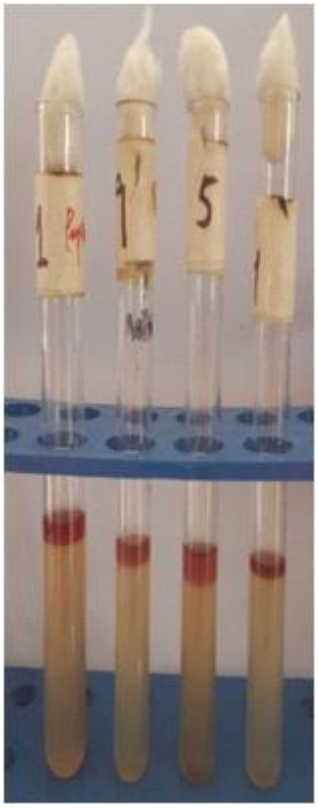

Plate 11: Indole test

Plate 12. Methyl Red Test

Plate 13. Voges-Proskauer Test

Plate 14: Citrate Utilisation Test

Plate 15: Triple Sugar Iron Test

Plate 16. Nitrate Reduction

Plate 17: Catalase test

Plate 18: Indole test

Plate 19: Methyl red test

Plate 20: Voges-Proskauer Test

Plate 21: Citrate test

Plate 22: Coagulase test

Plate 23: DNase test

Plate 24: Catalase test

Plate 25 : Oxidase test

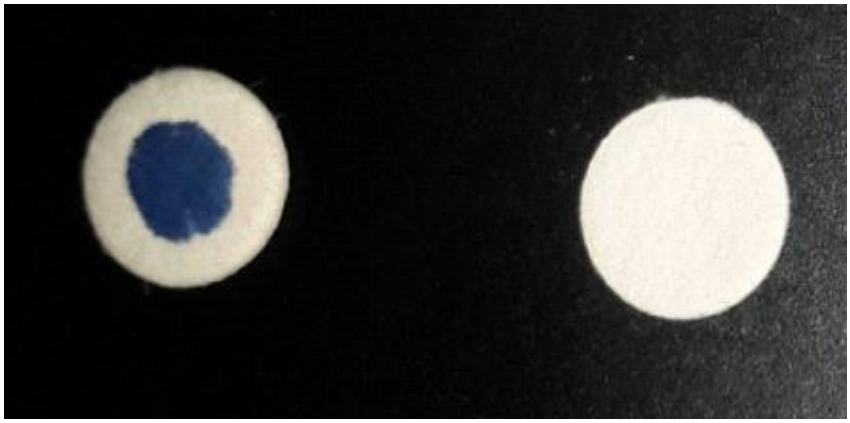

Plate 26 : Indoxyl acetate test

Plate 27 : Nitrate test

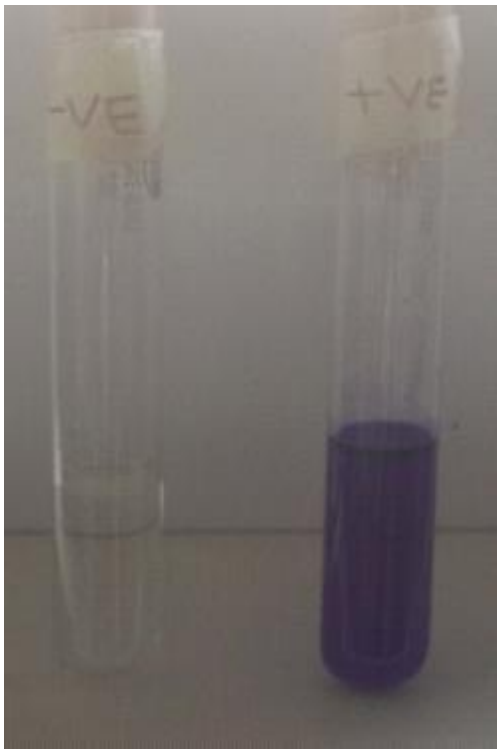

Plate 28 : Hippurate hydrolysis test

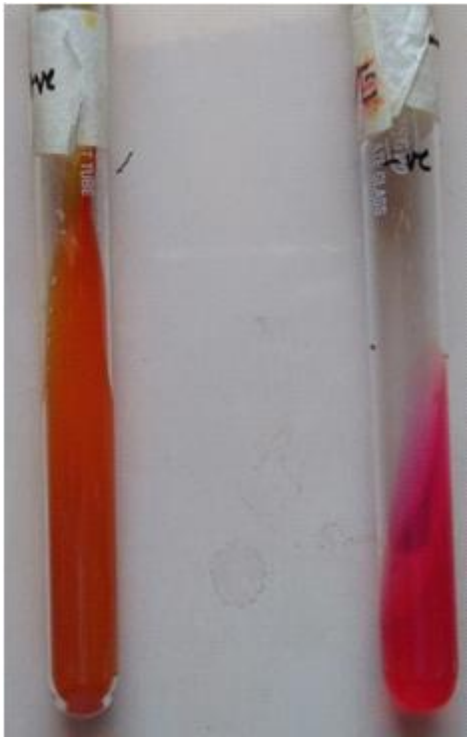

Plate 29 : Urease test

Plate 30 : Triple Sugar Iron test

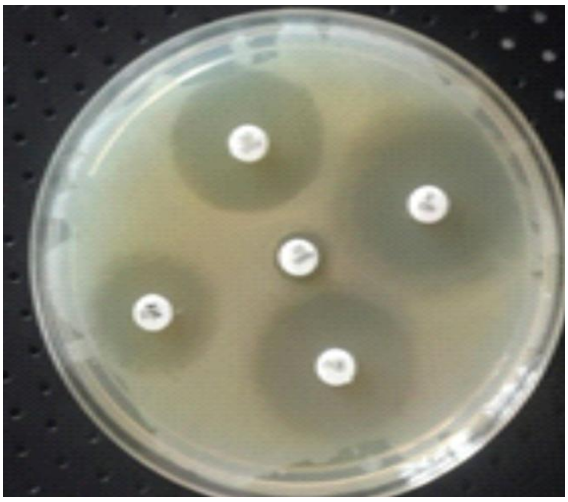

Plate 31: Antibiogram pattern of *E. coli*

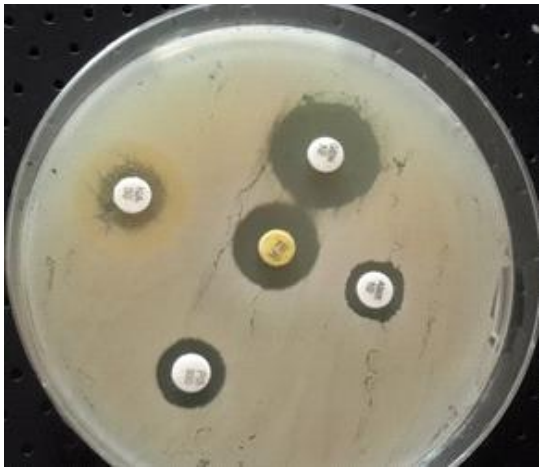

Plate 32 : Antibiogram pattern of *S. aureus*

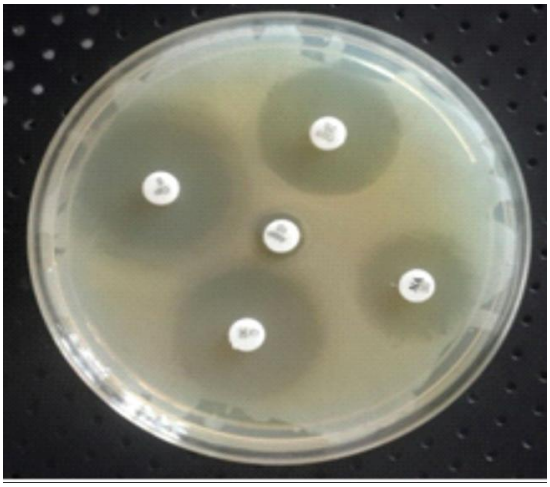

Plate 33 : Antibiogram pattern of *Campylobacter spp.*

Chapter – V

Discussion

CHAPTER – V

DISCUSSION

In the present study, a total of 75 mutton and 70 chicken meat samples were analysed to determine their microbiological quality and average total viable count were determined using standard procedures. The results of the present study are discussed in detail here.

The results pertaining to the standard plate count (SPC) of mutton samples (6.12±0.08) log_{10}cfu/g were nearly in agreement with the findings of Selvan *et al*., (2007) who reported SPC of 5.35±0.03log_{10}cfu/g. However, the results of the present study differ from the previous study conducted by Dabassa (2013) who **reported lower counts (3.67log_{10}cfu/g) in mutton.** The bacterial counts in the present study were generally high although they were below 10^7 where spoilage of meat occurs (Warriss, 2001). The higher counts could be due to the unhygienic practices followed during the meat handling and processing. Jeffery *et al*. (2003) revealed that the workers hand and equipments were the sources of contamination.

In the present study, average total plate count (6.17±0.08 cfu/g) **in the** chicken meat samples were found to be in agreement with the findings of Abu-Ruwaida *et al*. (1994) who reported total plate count ranging from 6.5 – 6.6 log_{10} cfu/g from the modern commercial slaughterhouses in Kuwait. However, much lower counts were reported by Lillard (1989), Willayat *et al*. (2006) Al Mohizea *et al*. (1994); Mead *et al*. (1993) and Khalifa with mean plate count of 3.71, 3.74, 4.67, 4.73 and 3.80-5.50 cfu/g, respectively in chicken carcasses. The average SPC in the samples examined appeared to be higher than permissible limit of 10^6cfu/g or 6log 10cfu/g (BIS, 1995). The higher total viable count of the poultry meat samples could be attributed to the inadequate maintenance of hygiene during the meat processing and handling time. Further the lack of

awareness of the butchers regarding the hygienic production of meat leads to contamination.

The results of the *Escherichia coli* count (ECC) of mutton samples in the present study were found to be 3.30± 0.55 $\log_{10}$cfu/g. Lower ECC was reported (2.0$\log_{10}$cfu/g) by Delmore (2000) The difference may be attributed to low maintenance of hygiene during processing of meat. The mean of ECC in chicken was 3.99±0.13 $\log_{10}$cfu/g in our study. Almost, similar observation were reported by Abu-Ruwaida *et al*. (1994) who found the values of 3.6 $\log_{10}$ cfu/g from the modern commercial slaughterhouses in Kuwait. Our result was in agreement with similar counts, 2.60 to 4.33$\log_{10}$cfu/g reported by Alvarez *et al*., (2002). The permissible limit for *E. coli* is 100cfu/g or 2$\log_{10}$cfu/g (BIS1995). The higher counts in the present study than the permissible limits are indicative of faulty methods of production, handling and storage.

In the present study out of 75 mutton samples, 23(30.6%) of the samples were found to be positive for *E. coli*. The percentages of positive samples obtained in the present study were higher than the previous reports by Mohammed *et al*. (2014) and Dabassa (2013) who had reported the percentage of positive samples to be below 20percent. Ahmad *et al*., (2013) and Yadav *et al*., (2001) reported higher percentage of positive samples (45% and 49%) respectively. This difference in the results may be attributed to different levels of hygiene practiced during the production and marketing. Upon analysis of 70 chicken meat samples, 29(41.4%) of the samples were found positive for *E. coli*. The findings of the present study are in agreement with the findings of Vazgecer *et al*., (2004) who reported 31percent of samples to be positive for *E. coli* in Ankara city, Turkey. The presence of *E. coli* in meat sample was indicative of possible contamination during slaughtering and processing and plant environment, contaminated water used for evisceration, improper bleeding and faecal contamination besides seasonal effects.

In the present study, *Staphylococcus aureus count* (SAC) for mutton samples was found to be 4.16±0.09$\log_{10}$cfu/gm. The results corroborate with the reports by Mohammed *et al*. (2013) who reported the count to be 4.2log10cfu/g. Similar results were obtained by Krishnaswamy *et al*. (1964) who reported the count to be 4.6 to 5.3

log10cfu/gm. The result of our study exceeded more than the permissible limit 1000cfu/g (BIS, 1995) which may be attributed to handling practices during slaughtering. In the present study, SAC for the chicken samples was found to be 4.16±0.09 cfu/g. Our results are in accordance with the results of Abu-Ruwaida *et al*. (1994) who reported the counts to be 4.1 $\log_{10}$ cfu/g of the chicken meat sample. The count in our study was slightly higher when compared to that of Alvarez *et al*. (2002) who reported the count to be 2.47 $\log_{10}$cfu/g and lower to that of Bachhil (1998) who reported total count 1.8x 10^5cfu/g. The result of our study exceeded more than permissible limit (1000cfu/g). Higher count in our study may be due to unhygienic practices adopted by butchers.

In the present study, we obtained 24 (32%) *Staphylococcus aureus* isolates from 75 mutton samples. Similar findings were Ashraf *et al*., (2015) *S. aureus* (27.08%) and Bhandare *et al*., (2010) (18.7%) from Mumbai. The result of our study corroborates with the study of Abd Abbas (2010) who detected 40percent *S. aureus* from Baghdad. In the present study, we obtained 25(35.7%) isolates *S. aureus* from 70 chicken samples. Our result were in agreement with that of Mawia *et al.,* (2011) who detected 37.65percent of *S. aureus* and Manso *et al*. (1987) who detected *S. aureus* 43.1percent. The presence of *S. aureus* in food is indicative of contamination from skin, mouth or nose of workers handling meat besides inadequately cleaned equipment may be source of contamination.

In the present study, none of the mutton sample (n=75) yielded *Campylobacter*. Our result was in agreement with that of Bailey *et al*. (2003) who could not isolate *Campylobacter* in mutton. Reason behind the lack of detection of *Campylobacter* isolates in mutton in our study could either be due to a low burden among the small ruminants. Higher percentage of occurrence of *Campylobacter* spp. in mutton was reported by Woldermariam *et al*. (2009) who detected 10.6percent from Ethiopia. The difference in our study could be due to the differences in hygienic conditions during cross contaminations that may occur during cutting of carcasses and unhygienic practices adopted by butchers.

In the present study we had obtained 21(30%) isolates of *Campylobacter* from 70 chicken samples. Our study was in agreement with that of Luu *et al*., (2006) who detected 31 percent occurrence of *Campylobacter* spp. However, our results were lower than that

of Chrystal *et al.*, (2008) who reported occurrence (44.8%) on poultry meat. The difference may be attributed to difference in defeathering, eviscerating, cutting carcasses in portions. Further in the present study out of 21(30%) isolates of *Campylobacter* we obtained 16(76.1%) occurrence of *C. jejuni* and 5(23.8%) *C. coli* from raw chicken samples. Our result was in agreement with that of Rahimi *et al*., (2010) who detected 76.4percent *C. jejuni* and 23.6percent *C. coli*. Nearly findings was observed by Akwuobu who detected (64%) *C. jejuni* and (23%) *C. coli* from raw chicken samples.. *Campylobacter* spp. in chicken may be due to unhygienic conditions, lack of scientific slaughter facilities, and faecal contamination during dressing of the carcasses, an unavoidable contamination in the retail shops. The present study clearly demonstrates the significance of poultry as reservoir of *Campylobacter*.

Based on the antibiogram pattern of *E. coli* (n=52), *E. coli* isolates revealed highest resistance to ampicillin (84.61%) while lowest resistance was shown against Ciprofloxacin (5.76%) and co-trimoxazole (5.76%). Our results were in corroboration with other workers who found higher resistance of *E. coli* against ampicillin, 100 percent by Hossneara *et al*. (2007); 98.02 percent by Saha *et al*. (2003) ; 77.5 percent by Dhanushree and Mallaya (2008). The resistance of *E. coli* isolate against above mentioned microbial agent may be due to indiscriminate and irrational use in the fields (Saha *et al.*, 2003).

All isolates of *Staphylococcus aureus* were found to be most sensitive to ciprofloxacin (91.83%) followed by amikacin (91.83%), polymixin B (83.67%) and chloramphenicol (73.46%). However, *S. aureus* isolates showed highest resistance against nalidixic acid (100%). Our result was in agreement with that of Park *et al*. (2011) who also recorded that the isolated *Staphylococcus aureus* strains from various raw foods in South Korea were all sensitive to amikacin. Overall, our result was in corroboration with that of other workers who reported low resistance of *S. aureus* against ciprofloxacin (Pereira *et al*. 2009; Uzeh *et al* .2006 and Khatoon *et al*. 2010). Higher resistance against nalidixic acid (100%) and lowest resistance against ciprofloxacin (96.37%) was also found by Mawia *et al*. (2011). The reason behind highest resistance may be due to indiscriminate use of antibiotics making gram positive bacteria resistant to that drug.

In the antibiotic resistance pattern of both *C. jejuni* and *C. coli* revealed the highest sensitivity against nalidixic acid (100%) and highest resistance (100%) was shown against cephalothin. Our result were found to be similar with that of Varma *et al*. (2000) who suggested that nalidixic acid can be used for the effective control of *Campylobacter* infection. The present study is in agreement with the findings of Akwoubo *et al*. (2010) who found resistance rates to cephalothin (84%) and Upadhyay *et al*. (2016) who found 100% resistance of isolate against cephaolthin. The high frequency of resistance of antibacterial agents in the study could be due to constant or indiscriminate use of these agents in poultry management in and partly due to beta- (β-) lactamase production by *Campylobacter* species (Sáenz *et al*., 2000). The resistant strains from other farm animals or farm environment can enter the contamination cycle to infect the animal and may increase antibiotic resistant population thereby posing threat to animal and human health (Piddock *et al*., 2000).

The present study indicated the count higher than permissible limit attributing to the unhygienic practices being followed during processing, handling of carcasses. The study revealed the presence of *E. coli*, *S. aureus* and *Campylobacter* in raw mutton and chicken. Their occurrence in raw meat indicates the unhygienic conditions during slaughtering, processing of meat. The wide antibiotic resistance pattern in the isolates pathogens is public health concern and could pose risk to consumers.

Chapter- VI

Summary and Conclusions

CHAPTER – VI

SUMMARY AND CONCLUSION

The Microbiological quality of meat and meat products is of public health significance. There are several reports on outbreaks of food borne illnesses because of consumption of meat. The meat gets contaminated from a variety of sources within and outside animal during the slaughter of animal and during its sale. In living animals, those surfaces in contact with the environment harbor a variety of microorganisms. The contaminating organisms are derived mainly from the hide of the animal and the faeces. The place of slaughter, the environment of the slaughter house the floor of the retail outlet, the air in the outlet and the vehicle used for the transport of the meat from the slaughter house to the retail outlet act as the external sources for the contamination of the meat. Foodborne pathogens have emerged to catch attention of researchers, food processers, consumers and all stakeholders and are very important cause of foodborne human disease. *Campylobacteriosis i*s described as an emerging foodborne disease (Houf and Stephan, 2007) and a major cause of bacterial gastroenteritis in humans (Kwan *et al.*, 2008).

In this study, a total of 145 samples comprising of 75 mutton and 70chicken meat samples were screened for microbiological contaminants. The results revealed that the mean value (log_{10}cfu/gm) of SPC, ECC and SAC of mutton were mutton (*n*=75) was found to be 6.12±0.08, 3.30± 0.55 and 4.08± 0.15 respectively while in chicken (n=70) SPC was found to be 6.17 ±0.05, ECC 3.99 ±0.13 and SAC 4.16± 0.09 respectively. SPC, ECC and SAC count in our study exceeded the permissible limit as per recommended standards (BIS, 1995; FSSAI, 2006)

A total of 52 samples were positive for *E. coli* which included 23 from mutton and 29 from chicken samples. A total of 49samples were positive for *Staphylococcus aureus* which include 24 from mutton and 25 from chicken whereas a total of 21 chicken samples were positive for *Campylobacter* spp. However, *Campylobacter* could not be isolated from mutton. Among 21 *Campylobacter* spp., 16 isolates were identified as *Campylobacter jejuni* and 5 isolates as *Campylobacter coli* morphologically and biochemically.

Overall from 145 raw meat samples, 122 (84.1%) bacterial isolates identified as *E. coli* 52(35.8%) *Staphylococcus aureus* 49(33.7%) and *Campylobacter* spp. 21 (30%). Highest number of isolates were from chicken 75 (51.72%) followed by mutton 47(32.41%). The present study revealed contamination of chicken meat was found higher than mutton. No occurrence of *Campylobacter* spp. was found in the samples of mutton. *C. jejuni* was found to be predominant species in chicken meat. The study indicates the presence of bacterial pathogens in raw meat which pose a risk to health of human.

The results of antibiotic sensitivity and resistance pattern of *E. coli* isolates revealed that ciprofloxacin was the most effective antibiotic against *E. coli* with 88.46percent sensitivity. In case of *S. aureus* with 91.83percent isolates were most susceptible to ciprofloxacin. Highest resistance of *E. coli* was found against Ampicillin being 84.61percent. All the isolates of *S. aureus* was most resistant to nalidixic acid with 100percent resistivity. *Campylobacter* was 100percent sensitive to nalidixic acid and 100percent resistant to cephalothin. Drug resistance due to indiscriminate use of antibiotics is emerging problem in food hygiene.

In Jammu city, sheep and poultry are slaughtered in large numbers on daily basis by local butchers under most unhygienic condition and our findings corroborate this fact as that mean values recorded for fresh meats were exceeded the standard acceptable microbial limits (BIS,1995; FSSAI, 2011). The poor hygienic conditions during processing and handling may expose the consumer to risk of pathogens. The microbial quality of meat sold in retail meat shops of Jammu city is not satisfactory.

On the basis of studies following could be concluded:

- The presence of known pathogens viz *S. aureus, E. coli* in mutton and chicken are of concern
- The presence of *Campylobacter* spp. in poultry meat poses a risk to consumers of this most popular food that needs remedial measures.
- The antibiotics resistance pattern further forewarns about the risk to consumers.

The result obtained in this study requiring the immediate measures for upgradation of the municipal slaughter houses and retail outlets/ shops besides the training of the personnel involved in the meat production and marketing chain.

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CERTIFICATE-IV

Certified that all the necessary corrections as suggested by the external examiner/evaluator and the Advisory Committee have been duly incorporated in the thesis entitled **"Evaluation of hygienic quality of raw meat (mutton and chicken) and characterization of isolated pathogens"** submitted by **Ms. Huma Kousar Zargar,** Registration No: **J-14-MV-381**.

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