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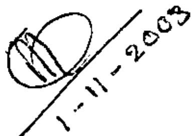

EVALUATION OF BACTERIOLOGICAL QUALITY OF BEEF CARCASSES IN MEAT PROCESSING PLANT

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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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2003

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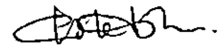
DECLARATION

I hereby declare that this thesis entitled **"EVALUATION OF BACTERIOLOGICAL QUALITY OF BEEF CARCASSES IN MEAT PROCESSING PLANT"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.



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CERTIFICATE

Certified that the thesis entitled "EVALUATION OF BACTERIOLOGICAL QUALITY OF BEEF CARCASSES IN MEAT PROCESSING PLANT" is a record of research work done independently by **Dr. C. Sethulekshmi**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	3
3	MATERIALS AND METHODS	34
4	RESULTS	53
5	DISCUSSION	63
6	SUMMARY	70
	REFERENCES	74
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1.	Mean total viable count of 40 beef samples belonging to Source A and B	53
2.	Distribution of carcasses based on total viable count	54
3.	Mean coliform count of 40 beef samples belonging to Source A and B	54
4.	Distribution of carcasses based on coliform viable count	55
5.	Mean *Escherichia coli* count of 40 beef samples belonging to Source A and B	55
6.	Distribution of carcasses based on *Escherichia coli* count	56
7.	Mean faecal streptococcal count of samples belonging to Source A and B	56
8.	Distribution of carcasses based on faecal streptococcal count	57
9.	Correlation coefficient between various bacterial count of the samples belonging to Source A	57
10.	Correlation coefficient between various bacterial count of the samples belonging to Source B	58
11.	Pathogenic organism isolated from beef carcass samples	59
12.	Distribution of *Escherichia coli* serotypes isolated from the carcass samples	60
13.	Mean bacterial load of processing equipments, ice, water and hand washings	61
14.	The mean bacterial load of air samples in processing plant	62

LIST OF FIGURES

	Title	Between pages
Plate 1.	*Sampling sites on beef carcasses*	34&35
Fig.1.	Distribution of carcasses based on total viable count	54&55
Fig.2.	Distribution of carcasses based on coliform count	54&55
Fig.3.	Distribution of carcasses based on *Escherichia coli* count	57&58
Fig.4.	Distribution of carcasses based on faecal streptococcal count	57&58

Introduction

1. INTRODUCTION

The interdependence between man and animals for their existence may be as old as the origin of human being on this planet. Livestock and poultry not only provide valuable foods but also play a very important role in the rural economy in terms of providing employment opportunity, income generation, earning of foreign exchange, sustainable agriculture and so forth.

India is one of the developing countries with the highest livestock population in the world. The meat trade of the country depends on 219.6 million cattle, 94.1 million buffaloes, 58.2 million sheep, 123.5 million goats, 17.5 million pigs and 413 million chickens. Though the country has the highest livestock population, its meat production is too low. During the year 2000, the country had produced only 4.7 million metric tonnes. However, an increasing trend in the meat production of the country was observed during the past two decades.

In India meat is primarily produced in about 3600 registered slaughterhouses, which are under the control of local bodies. The infrastructure and other facilities available in these slaughterhouses are quite unsatisfactory and the hygienic practices followed in these abattoirs are far below from satisfactory. However, during early1970's, eight bacon factories were started in different states of the country and majority of these plants were closed, due to lack of clear vision, and sound policy. Now the country have 24 modern abattoirs and meat processing plant under private sector which are in operation, to cater the needs of the export market.

During the slaughter and dressing of food animals contamination of the carcasses occurs from the animal's hide, intestinal content, personnel engaged in these processes and also from contaminated equipment and environment. Contamination of carcasses with bacterial organisms from these sources has great significance in meat trade and is depended on the level and type of bacterial contamination. The bacterial contamination not only differs between carcasses

produced in a plant but also differ between carcasses produced from different meat processing plants.

The bacterial contaminants from the various sources include both saprophytic and pathogenic organism. The growth and multiplication of the former group of organism cause spoilage of the valuable meat and shortens the shelf life of meat which may also depends on the initial bacterial load of carcasses. The latter group of organisms is responsible for the causation of food borne infection and intoxication but their growth and multiplication do not produce appreciable change in meat. Considering all the above facts the present study was undertaken with the following objectives.

1. To evaluate the bacterial quality of carcasses by estimating
   - a. Total viable count (TVC)
   - b. Coliform count (CC)
   - c. *Escherichia coli* count (ECC)
   - d. Faecal streptococcal count (FSC)
2. To detect the presence of bacteria of public health significance such as
   - *a. Escherichia coli*
   - *b. Staphylococcus aureus*
   - c. Salmonella
   - *d. Listeria monocytogenes*
3. To assess the role of environment, processing equipment and meat plant operators by estimating the bacterial load of the samples as indicated in the first objective.

Review of Literature

2. REVIEW OF LITERATURE

The growth and multiplication of microorganism not only results in the spoilage of meat and its products but also is also associated with food borne infection and intoxication in the consumers. Therefore, frequent microbial quality evaluation of the carcasses as well as meat is of primary concern in the prevention of spoilage of meat and its product, and also to safeguard the consumer health. Hence, the present study was undertaken to evaluate the bacterial quality of beef carcasses selected from a meat processing plant.

2.1. BACTERIAL COUNT

2.1.1. Total Viable Count

Stringer *et al.* (1969) investigated the microbial level of 180 beef carcasses collected from a packing plant immediately after slaughter. The study revealed that the logarithmic mean microbial population per square inch was 4.70. The mean microbial count of inside neck was 4.68 log/in^2. In chine bone area, the average microbial population was 4.54 log/in^2, while the mean population in exposed area of the clod was 4.74 log/in^2. Average microbial count in exposed muscle above aitch-bone and fat on outside of round was 4.82 log/in^2 and 4.08 log/in^2, respectively.

Rao (1970) assessed the bacteriological quality of 15 ground beef samples obtained from local retail market, one from meat laboratory of Kansas State University and four from military organizations. The study revealed that the standard plate count of the samples obtained from the local retail market ranged from 11,000 to 36,533,000/g. The count of the sample from the meat laboratory was 10,230,000/g. The count in samples belonging to military organization ranged between 8000 and 61,600/g.

Kotula *et al.* (1974) evaluated the bacterial quality of 96 beef carcasses washed with water containing 200-ppm chlorine. The study revealed that average total aerobic count on the forequarters was about 3,000/cm^2 before wash and 100/cm^2 45 minutes after wash and 27/cm^2 24 h after wash.

Chambere *et al.* (1976) studied the microbial quality of 459 unfrozen ground beef samples consisting of 232 from independent retail stores and 227 from supermarket. The samples belonging to the former source had a mean total plate count of 6.8590 $\log_{10}$/g and the count of the samples from the later source was 6.8959 $\log_{10}$/g.

Hankin *et al.* (1976) examined 22 ground beef samples collected from retail markets and found that total number of bacteria ranged from 24,000 to 30,000,000/g of sample.

Seideman *et al.* (1976) studied the microbial quality of wholesale and retail beef cuts which included 45 beef knuckles, 45 beef ribs and 16 beef chucks during storage at 1-3° C. The mean mesophilic count of beef knuckles on zero day was 2.14 $\log_{10}$ /in^2. The count on beef ribs was 2.73 $\log_{10}$ /in^2 and that on beef chucks was 2.39 $\log_{10}$ /in^2.

Lazarus *et al.* (1977) compared the efficiency in recovering the bacterial flora from round, rib, aitch bone, fore shank and neck of six beef carcasses by moist swab contact method and tissue removal rinse technique. They also compared the efficiency of these methods in recovering bacteria from the neck area of 26 beef carcasses, collected from University of Florida abattoir. The overall mean total microbial count recovered by moist swab method from the five sites of six beef carcasses was 3.66 $\log_{10}$/6.45 cm^2 and the corresponding count obtained by tissue rinse method was 3.35 $\log_{10}$ /6.45 cm^2. The counts obtained from neck area of 26 beef carcasses by moist swab and tissue rinse methods were 3.20 and 3.24 $\log_{10}$/6.45 cm^2 respectively.

Rao (1978) evaluated the keeping quality of dried and minced beef at 48 h post mortem, under commercial conditions, held at 4 to $6^0C \pm 2^0$. The study revealed that the initial load of aerobic organism was 2.5×10^{8}/g.

Emswiler and Kotula (1979) evaluated the bacteriological quality of hot and chilled ground beef stored at 0°C for 45days. Hot boned ground beef sample had an aerobic count of 5.13 $\log_{10}$/g.

Ali and Vanduyne (1981) analysed the microbial quality of six lots of fresh ground beef purchased at intervals from January to June from a local supermarket. The samples had a mean aerobic plate count of 6.35 ± 0.41 $\log_{10}$/g.

Jay and Margitic (1981) determined the bacteriological quality of 111 fresh ground beef samples collected from 45 retail supermarkets. The mean aerobic plate count of the samples was 9.50×10^6/g.

Nortje and Naude (1981) evaluated the bacterial quality of ten carcass sites on each of 156 beef carcasses. The study revealed that the samples had a mean aerobic count of 4.5×10^2/ cm^2.

Kuttinarayanan and Soman (1985) assessed the hygiene quality of market beef samples collected from the slaughter house attached to the College of Veterinary and Animal Sciences, Panchayat market meat stall at Mannuthy, Meat Stall at East Fort and West Fort, and reported that the samples had a mean aerobic count of $36.4 \pm 3.03 \times 10^6$, $65.03 \pm 3.91 \times 10^6$, $89.2 \pm 3.77 \times 10^6$ and $94.48 \pm 5.46 \times 10^6$/g, respectively.

Bhadekar *et al.* (1986) evaluated the microbial quality of six buffalo meat cuts and reported that the samples had an average aerobic plate count of 7.56 log cfu /g.

Hudson *et al.* (1986) assessed the bacteriological quality of minced beef purchased from seven supermarkets and eleven butcher's shops. The samples

from the former source had mean total viable count of 5.62 $\log_{10}$/g and the corresponding count of the samples from the latter source was 5.72 $\log_{10}$/g.

Scriven and Singh (1986) analysed microbial load on retail samples of beef mince and beef rump. They reported that the mean total plate count of retail cuts of beef rump was 4.6×10^6 cells/g and the count on beef mince was 5.0×10^7 cells/g.

Das *et al.* (1988) investigated bacteriological quality of 14 buffalo meat samples collected from corporation slaughter house and reported that the samples had a mean total aerobic plate count of log 5.45 ± 0.052/g.

Lasta and Fonrouge (1988) evaluated the efficiency of two sampling areas of 10 cm^2 and 100 cm^2 of the bovine carcass to assess the hygienic status of abattoirs during the slaughter process. The study revealed that the bacterial counts below 100 cm^2 area did not prove to be an adequate indicator of the hygienic levels of the slaughtering process. The mean total viable count of samples from one of the slaughterhouses was 2.07 $\log_{10}/cm^2$ and for the other slaughterhouse was 2.09 $\log_{10}/cm^2$, when 100 cm^2 area of the brisket was sampled.

Dixon *et al.* (1991) studied the microbiological quality of beef carcasses handled under strict sanitary conditions and treated with lactic acid and carcasses handled under conventional condition. They observed that the aerobic plate count of the carcasses belonging to the former group ranged from <0.2 to 1.2 $\log_{10}/cm^2$ and the count in the latter group ranged between 2.8 and 3.8 $\log_{10}/cm^2$.

Okodugha and Aligba (1991) conducted a study on microbiological quality of seven raw beef samples obtained from Irrua market and reported that two samples had a mean aerobic plate count of 6.34 ± 0.26 $\log_{10}$/g.

Lasta *et al.* (1992) assessed the bacterial status of 523 beef carcasses collected from six abattoirs viz., A, B, C, D, E and F. The mean mesophilic count

on beef carcasses obtained from the B and E abattoirs was 1.92 log cfu/cm^2, whereas the count of the samples obtained from the sources C and F was 2.19 log cfu/cm^2.

Lefebvre *et al.* (1992) examined ground beef purchased from local supermarkets and reported that the samples had a mean mesophilic count of 5.6×10^5 cfu/g.

Barkate *et al.* (1993) evaluated the effect of hot water spray on the bacteriological quality of beef carcasses by estimating the aerobic plate count of 29 samples each of pre sprayed and post sprayed groups and reported that the former group of samples had a mean aerobic plate count of 2.4 log_{10} /cm^2 and the count on the latter group was 2.3 log_{10} /cm^2.

Latha (1993) evaluated the effect of acetic acid and propionic acid at one and two per cent strength as sanitizer on beef samples collected from Kerala Agricultural University slaughterhouse. The mean total viable count of control samples of acetic acid treated group was 4.85 ± 0.034 log_{10} cfu/cm^2, while the count on the control samples of propionic acid treated group was 4.77 ± 0.025 log_{10} cfu/cm^2.

Tarwate *et al.* (1993) examined 14 different sites each, from seven buffalo carcass samples and reported that mean total viable count of carcass sites was 4.70 ± 0.40 log cfu/cm^2.

Kotula and Thelappurate (1994) assessed the effect of application of lactic acid and acetic acid on microbiological quality of 20 beef cuts obtained from anterior region of the rib eye. The study revealed that the untreated samples had a mean total count of 5.8 ± 0.08 log cfu/g.

Rajeev (1994) evaluated the effect of lactic acid treatment on bacterial load on beef samples collected from University slaughterhouse. The study

revealed that the samples had an initial mean total viable count of 4.38 ± 0.01 log cfu/cm^2.

Ziauddin *et al.* (1994) studied the bacteriological quality of 24 buffalo carcasses, 12 each from two different slaughter units. From each carcass the samples were collected from four sites viz., neck, shoulder, loin and leg. The study revealed that the samples from one of the slaughter units had a mean total plate count of 5.06 ± 0.42 $\log_{10}/cm^2$ and the count of the samples from the other slaughter unit was 4.87 ± 0.41 $\log_{10}/cm^2$.

Kenney *et al.* (1995) studied the effect of various spray treatments on microbial load of 15 steers slaughtered at Kansas State University abattoir. They reported that, before treatment the neck and sternum had mean aerobic plate counts of 2.50 and 3.22 $\log_{10}$ cfu/cm^2, respectively. Following treatments the counts at the two sites were 2.07 and 2.25 $\log_{10}$ cfu/cm^2, respectively.

Prasai *et al.* (1995) compared the effect of trimming and washing treatments on total aerobic bacterial population of 24 beef carcasses obtained from a large commercial processing plant. The carcasses without trim and wash treatments had highest aerobic plate count of 3.5 log cfu/cm^2, whereas, treatment trim but no wash had lowest count of 0.5 log cfu/cm^2, followed by trim and wash and no trim but wash in which the counts were 2.6 and 3.2 log cfu /cm^2, respectively.

Gill *et al.* (1996) assessed the microbiological quality of samples collected from hock, butt, anal area, rump, caudal back, waist, cranial back, neck, caudal brisket and cranial brisket of 25 beef carcasses selected randomly from a beef packing plant. The mean log total count per cm^2 of these carcass sites after washing were 2.48, 2.39, 1.67, 2.40, 1.92, 1.92, 2.77, 2.44, 3.06 and 2.34, respectively.

Hygienic condition of 24 meat samples each, collected from six carcass breaking plants A, B, C, D, E and F were examined by Gill *et al.* (1996). The

mean total aerobic counts of samples belonging to plants A, B, C, D, E and F were 2.89, 3.60, 3.87, 4.16, 4.10 and 4.26 $\log_{10}$/g, respectively.

Karr *et al.* (1996) evaluated the microbiological quality of 40 beef carcasses each, obtained from two commercial slaughter and processing operations and reported that the samples had an aerobic plate count in the range of 11 to 10^6 cfu/ cm^2. The count on 70 per cent of carcasses ranged between 10^3 and 10^4 cfu/cm^2.

The bacterial flora on 12 beef carcass loin stored in vacuum and 100 per cent CO_2 at 2 and 6°C and on five beef carcass loin stored in vacuum and different mixtures of CO_2 and N_2 at –1 and 2°C were determined in two experiments (Nissen *et al.* 1996). The mean total microbial count on loin before storage in experiment one was 1.9 ± 0.3 $\log_{10}$ cfu/cm^2 and that in experiment two was 2.9 ± 0.6 $\log_{10}$ cfu/cm^2.

Dorsa *et al.* (1997) compared the efficiency of sponge sampling and excision sampling methods on the aerobic bacterial count from 30 beef carcass samples. The study revealed that the mean total aerobic plate count in the samples collected from post wash carcasses by sponge sampling method was 1.6 $\log_{10}$ cfu/ cm^2 and the corresponding count of the samples taken by excision method was 2.1 $\log_{10}$ cfu/cm^2.

Eisel *et al.* (1997) evaluated the microbial load on fresh meat samples taken from brisket, skirt, round and flank of beef carcasses and also from the boxed beef, ground beef and retail cuts. The mean aerobic plate counts for brisket, skirt, round and flank were 6.9, 6.6, 4.7 and 4.0 $\log_{10}$ cfu/g, respectively. The boxed beef had a mean aerobic plate count of 6.4 $\log_{10}$ cfu/g. The count for both coarse and fine ground beef samples was 4.7 $\log_{10}$ cfu/g. The average aerobic plate count for retail cuts was 3.0 $\log_{10}$ cfu/g.

Gill and Bryant (1997) assessed the hygienic performance of the beef carcass cooling process of two plants A and plant B, on 25 beef carcasses that

entered and left the chiller. The study revealed that the carcasses entering and exiting the chiller in plant A had mean total aerobic counts of 3.06 and 3.04 log_{10}/cm^2, respectively. The corresponding counts of the carcasses entering and exiting the chiller in plant B were 2.35 and 1.87 log_{10}/cm^2.

Linton *et al.* (1997) compared the efficiency of recovery of micro-organism from 104 fresh and frozen beef samples collected from mid-western retail ground beef processing plants, using conventional pour plate technique and petrifilm method. In the pour plate method, the average mesophilic bacterial count of the fresh and frozen samples was 3.77 ± 1.02 log cfu/g and in the petrifilm method the samples had a mean count of 3.55 ± 1.02 log cfu/g.

Rahkio and Korkeala (1997) examined 32 beef carcasses collected from four slaughterhouses to determine the level of airborne bacteria on beef carcasses and the association between the airborne bacteria and the carcass contamination. The study revealed that the beef carcass contamination in the slaughterhouse varied from 1.44 to 2.61 log cfu/cm^2.

Prasai *et al.* (1997) evaluated the effect of lactic acid spray and storage of beef carcass loin. The study revealed that the control samples on zero day of storage had a mean aerobic plate count of 3.1 ± 0.4 log cfu/cm^2.

Hinton *et al.* (1998) assessed the bacteriological quality of 2200 samples obtained from the surface of beef carcasses. The proportions of samples with an aerobic plate count in the categories of excellent, good, fair, poor and bad were 3, 37, 27, 27 and 6 per cent respectively.

Sofos *et al.* (1999) analysed the microbiological quality of 3,780 beef carcass samples from seven beef slaughter units, and reported that the aerobic plate count in 46.7 to 93.3 per cent of beef carcass samples was $\leq 10^4$ cfu/cm^2.

Bacon *et al.* (2000) studied the microbial quality of 1,280 beef carcasses at both hide on and hide off location. The study revealed that the hide removal process led to the introduction of a mean total plate count of 7.6 log cfu/100 cm^2.

Cheah and Gan (2000) determined the effect of galangal extract on microbial stability of locally available raw minced beef samples stored at 4 ± 1°C. The study revealed that the untreated samples had a mean total plate count of 5.74 ± 0.05 log_{10} cfu/g.

Gill and Jones (2000) studied the efficiency of recovery of bacteria by swabbing with cotton wool on selected sites of 25 dressed beef carcasses, each collected from the beef packing plants B and C, and 25 cooled beef carcasses, each obtained from plants B and D. During the study it was observed that the dressed beef carcasses from plants B and C had mean aerobic plate counts of 1.56 and 2.06 log_{10}/cm^2, respectively. The corresponding count of cooled carcass from plants B and D were 1.47 and 2.09 log_{10}/cm^2.

Jericho *et al.* (2000) analysed the microbiological quality of 60 boxed beef cuts collected from highline speed abattoir and reported that the samples had a mean aerobic bacterial count of 2.87 log_{10} most probable number growth units/cm^2.

Jolly (2000) studied the bacterial quality of 75 market beef samples procured from retail outlets located at four different regions. The study revealed that the samples had an overall mean total viable count of 7.39 ± 0.06 log_{10} cfu/g.

Wyss (2000) assessed the hygienic levels of 100 veal carcasses and reported that one fourth of the samples from forequarters and hindquarters had total microbial count under 2000 cfu/cm^2.

Gill *et al.* (2001) compared the efficiency of recovery of bacteria by excision and swabbing on 25 beef loin primal cuts collected from a beef packing plant. The cuts sampled by excision method had a mean total aerobic count of

3.86 log_{10}/cm^2 on fat surfaces, but the corresponding counts of samples collected by swabbing with cotton wool, sponge and gauze were 3.03, 3.59 and 3.16 log_{10}/cm^2, respectively. The samples obtained from lean surface by excision, swabbing with cotton wool, sponge and gauze had mean total aerobic counts of 2.81, 2.78, 2.56 and 2.30 log_{10}/cm^2, respectively.

Hansson (2001) conducted microbiological analyses of 200 samples taken from beef loin and beef sternum. The samples from the high capacity slaughterhouse had a mean aerobic microorganism of 2.59 log_{10} cfu/cm^2 and the count of the samples from low capacity slaughterhouse was 3.44 log_{10} cfu/cm^2.

Murray *et al.* (2001) assessed the hygienic quality of beef carcasses collected from seven European Union – approved abattoirs in Northern Ireland at 24 to 48 h after kill. The mean total viable count of samples at 22°C of incubation was 2.75 ± 0.64 log cfu/cm^2.

Philips *et al.* (2001) evaluated the microbiological quality of beef carcass samples collected from export (812), domestic (273) and very small establishment (190). It was observed that the samples collected from export had mean total viable count of 2.20 log_{10}/cm^2. The counts of the samples from domestic and very small establishments were 2.61 and 3.10 log_{10}/cm^2, respectively.

Skandamis and Nychas (2001) examined the influence of various packing and storage conditions on the growth of spoilage bacteria on minced beef. The study revealed that the control samples had a mean total viable count of 6.58 ± 0.06 log_{10} cfu/g.

Martinez *et al.* (2002) examined bacterial load on neck, brisket and renal sites of 67 bovine carcasses collected from two bovine slaughterhouses. The mean aerobic plate counts on neck, brisket and renal sites after washing were 3.8, 3.4 and 3.4 log cfu/cm^2 and that after skinning were 1.7, 1.8 and 1.9 log cfu/cm^2,

respectively. The counts at these sites after splitting were 4.1, 3.4 and 3.5 log cfu/cm^2, respectively.

Mathew *et al.* (2003) evaluated the bacterial quality of 100 market beef samples collected from retail shops located at East Fort, West Fort, Sakthanthampuran market and Mannuthy market. The study revealed that the mean total viable counts of samples collected from these areas were 6.61 ± 0.15, 6.68 ± 0.17, 6.84 ± 0.19 and 6.49 ± 0.15 $\log_{10}$ cfu/g, respectively.

2.1.2. Coliform Count

Rao (1970) examined 15 ground beef samples obtained from local retail market, one sample from the meat laboratory of Kansas State University and four samples from a military organization. The study revealed that the coliform count in the samples from the local retail market ranged from 233 to 71,333/g. The count of the sample obtained from the meat laboratory was 3866/g and the count of the samples collected from military organization ranged between zero and 550/g.

Chambere *et al.* (1976) examined 232 ground beef samples purchased from independent retail store and 227 from supermarket. The former samples had a mean coliform count of 2.6535 log/g and the count in the latter samples was 2.8005 log/g.

Shoup and Oblinger (1976) evaluated microbiological quality of 40-retail ground beef samples prepared in centralized operation and in four local stores. They reported that only three of the 40 samples of ground beef showed levels of less than 100 coliforms/g (MPN/g).

Rao (1978) examined dried minced beef held at refrigeration temperature of 4 to 6°C ± 2 and found that the samples were free of coliforms.

Emswiler and Kotula (1979) evaluated bacteriological quality of ground beef chubs prepared from fresh hot boned and cold boned meat. They reported that the average coliform count in fresh hot boned beef was 12 MPN/g.

Ali and Vanduyne (1981) evaluated six lots of fresh ground beef collected from local supermarket and reported that the samples had a mean coliform count of 4.48 ± 0.24 $\log_{10}$/g.

Kuttinarayanan and Soman (1985) reported a mean coliform count of $2.91 \pm 0.37 \times 10^5$/100 g on meat samples collected from slaughter house attached to College of Veterinary and Animal Sciences and $13.52 \pm 2.24 \times 10^5$/100g from meat stall in Panchayat market at Mannuthy. The count in the samples from East Fort and West Fort meat stalls were $45.75 \pm 13.94 \times 10^5$/100g and $77.60 \pm 16.4 \times 10^5$/100 g, respectively.

Scriven and Singh (1986) determined the coliform count of retailed minced beef samples and recorded that the count ranged from seven to 50,000/g, with a mean count of 1159/kg, whereas the count of retailed beef rump samples ranged from zero to 70/g, with a mean count of 11/g.

Okodugha and Aligba (1991) recorded that mean coliform count of raw beef samples collected from Irrua market was 4.95 ± 0.87 $\log_{10}$/g.

Lasta *et al.* (1992) assessed the bacterial status of 520 beef carcasses collected from six abattoirs .The study revealed that faecal coliform counts were lower than 1 cfu/cm^2 on all carcasses from abattoirs scored as "very good" and in 6.8 per cent of samples from abattoir graded as "good".

Latha (1993) obtained a mean coliform count of 2.67 ± 0.44 $\log_{10}$ cfu/cm^2 on the acetic acid untreated beef samples collected from Kerala Agricultural University slaughter house and the corresponding count for the propionic acid untreated beef sample was 2.47 ± 0.20 $\log_{10}$ cfu/cm^2.

Tarwate *et al.* (1993) reported that faecal coliform count of 14 different carcass sites each, from seven buffalo carcass surfaces ranged from 3.26 ± 0.26 to 5.14 ± 0.36 log cfu/cm^2.

Rajeev (1994) assessed the effect of lactic acid treatment on bacterial load on beef samples and reported that the samples had an initial mean coliform count of 2.58 ± 0.03 log cfu/cm^2.

Ziauddin *et al.* (1994) examined leg, loin, shoulder and neck of 24 buffalo carcasses collected from two slaughter units. They found that the overall mean coliform count of the samples belonging to one of the slaughter units was 3.43 ± 0.28 log_{10} cm^2 and the count of the samples from the other unit was 3.21 ± 0.28 log_{10}/cm^2.

Prasai *et al.* (1995) examined 48 beef carcasses which were subjected to no trim and no wash, trim but no wash, trim and wash and no trim but wash treatments and found that the coliform count between treatments ranged from 0.03 to 0.4 log cfu/cm^2.

Gill *et al.* (1996) assessed the microbiological quality of samples collected from hock, butt, anal area, rump, caudal back, waist, cranial back, neck, caudal brisket and cranial brisket of 25 beef carcasses selected randomly from a beef packing plant. The mean log_{10} coliform counts/100 cm^2 of the carcass sites after washing were 0.65, 0.95, 1.11, 1.68, 0.67, -0.01, 0.90, 0.76, 0.65 and 0.59, respectively.

Gill *et al.* (1996) assessed the hygienic conditions of each of 24 beef carcass samples collected from six carcass breaking plants A, B, C, D, E and F. They reported that the mean coliform counts of samples belonging to A, B, C, D, E and F were 0.09, 1.00, 1.65, 0.60, 1.65 and 2.40, respectively.

Karr *et al.* (1996) studied the microbiological quality of 40 beef carcass samples and recorded that 47.5 per cent of samples had a total coliform count between 10^1 and 10^3 cfu/cm^2.

Nissen *et al.* (1996) determined the bacterial flora on 12 beef carcasses stored in vacuum and 100 per cent CO_2 at 2 and 6^0C and in five beef carcasses stored in vacuum and different mixtures of CO_2 and N_2 at –1 and 2^0C. They reported that mean coliform count on 12 beef carcasses before storage was 0.1 $\log_{10}$ cfu/cm^2 and the corresponding count on five beef carcasses before storage was 1.0 ± 0.8 $\log_{10}$ cfu/cm^2.

Eisel *et al.* (1997) assessed the microbial load on brisket, skirt, round and flank region of beef carcasses and also boxed beef, ground beef and retail cuts. They reported that coliform count ranged from 1.4 to 3.2 $\log_{10}$ cfu/g, with higher counts on samples from boxed beef and the brisket area of the beef carcass.

Gill and Bryant (1997) evaluated the efficiency of carcass cooling process on each of 25 randomly selected carcasses entering and leaving chillers at two beef slaughtering plants. The study revealed that the mean coliform count of the carcasses entering the chiller of one of the two plants was 1.25 $\log_{10}$/100 cm^2 and the count on the carcasses leaving the chiller was –0.46 $\log_{10}$/cm^2. The corresponding count of samples of the other plant was 1.13 and –0.23 $\log_{10}$/100 cm^2, respectively.

Linton *et al.* (1997) compared the efficiency of pour plate method and petrifilm methods in recovering microorganism from 77 fresh and frozen beef samples collected from mid-western ground beef processing facility. They reported that mean coliform count recovered by pour plate method was 2.43 ± 0.77 log cfu/g and that by petrifilm method was 2.43 ± 0.80 log cfu/g.

Sofos *et al.* (1999) determined the microbiological load on 3,780 beef carcass samples obtained from seven beef slaughtering plants and reported that the total coliform count ranged between 0.13 and 0.68 log cfu/cm^2.

Bacon *et al.* (2000) examined 1,280 beef carcass samples collected from eight beef packing plants. The study revealed that the total coliform count of the samples ranged between 3.0 and 6.0 log cfu/100 cm^2.

Gill and Jones (2000) studied the efficiency of recovery of bacteria by swabbing with cotton wool on selected sites of 25 dressed beef carcasses, each collected from the beef packing plants B and C and 25 cooled beef carcasses each obtained from the plants B and D. During the study, it was observed that the dressed beef carcasses from the plants B and C had a coliform count of 1.52 and 2.29 log_{10}/125 cm^2, respectively. The cooled samples from plant D had a count of 0.60 log_{10}/125 cm^2.

Jericho *et al.* (2000) assessed the microbiological quality of 60 boxed beef cuts collected from high-line speed abattoir and reported that the samples had a mean coliform count of 0.99 log_{10} most probable number growth units/cm^2.

Jolly (2000) assessed the bacterial quality of 75 market beef samples collected from four different regions and reported that the samples had an overall mean coliform count of 4.62 ± 0.06 log_{10} cfu/g.

Gill *et al.* (2001) obtained mean coliform counts of 2.97, 2.01, 2.73 and 1.90 log_{10}/100 cm^2 by excision, swabbing with cotton wool, sponge and gauze respectively, on randomly selected sites on fat surfaces of 25 beef loin primal cuts. The corresponding counts of the samples obtained from lean surfaces by excision, swabbing with cotton wool, sponge and gauze were 2.73, 2.07, 2.32 and 2.09 log_{10}/100 cm^2, respectively.

Hansson (2001) evaluated microbiological quality of 200 samples of beef carcasses taken from beef loin and beef sternum collected from four high capacity and four low capacity slaughterhouses. The study revealed that the highest value of coliform bacteria on beef from high capacity abattoirs was 370 bacteria/cm^2 and from low capacity abattoirs was 15,000 coliform bacteria/cm^2.

Martinez *et al.* (2002) evaluated the bacterial load on neck, brisket and renal sites (flank) of 67 bovine carcasses after skinning, splitting and washing .The study revealed that after washing these sites had mean coliform counts of 3.0, 2.9 and 2.4 log cfu /cm^2 respectively.

Mathew *et al.* (2003) examined 100 market beef samples collected from four regions and reported the overall mean coliform count of the samples as 2.73 ± 0.09 log_{10} cfu/g.

2.1.3. Escherichia coli Count

Shoup and Oblinger (1976) examined 40 retail samples of ground beef and reported that only one centrally prepared sample exceeded an *Escherichia coli* level of 50 MPN/g.

Emswiler and Kotula (1979) estimated the *Escherichia coli* count of hot and chilled ground beef stored at 0^0C for 45 days. They reported that the organism was absent in fresh hot boned beef.

Kuttinarayanan and Soman (1985) reported that the mean *Escherichia coli* count of beef samples collected from the slaughter house attached to the College of Veterinary and Animal Sciences was 20.5 ± 3.0 × 100/g. The count of the samples obtained from meat stall in Panchayat market at Mannuthy was 35.27 ± 1.9 × 100/g. The corresponding count of the samples from East Fort and West Fort were 40.35 ± 3.64 × 100/g and 53.24 ± 4.26 × 100/g, respectively.

Hudson *et al.* (1986) analysed minced meat from seven supermarkets and eleven butcher's shops. The samples from the former source had a mean *Escherichia coli* biotype I count of 1.72 log_{10}/g and the count on the samples from the latter source was 1.76 log_{10}/g.

Kotula and Thelappurate (1994) evaluated the effect of lactic acid and acetic acid on microbiological quality of 20 beef cuts obtained from anterior

region of the rib eye. The study revealed that the untreated samples had a mean *Escherichia coli* count of 5.3 ± 0.07 log cfu/g.

Prâsai *et al.* (1995) evaluated the effect of trimming and washing treatments on bacterial population of 24 beef carcasses obtained from a large commercial processing plant. They reported that the mean *Escherichia coli* count was higher in no trim no wash treatment than in rest of the treatments. The count between treatments ranged from 0.04 to 0.2 log cfu/cm^2.

Gill *et al.* (1996) reported that 25 beef carcasses selected randomly from a beef packing plant had mean *Escherichia coli* count of 0.44, 1.00, 0.93, 1.65, 0.69, –0.14, 0.69, 0.64, 0.50 and 0.55 log/100 cm^2 for samples obtained from hock, butt, anal area, rump, caudal back, waist, cranial back, neck, caudal brisket and cranial brisket respectively.

Gill *et al.* (1996) examined 24 beef samples obtained from six carcass breaking plants A, B, C, D, E, and F and reported that the samples from these plants had mean *Escherichia coli* counts of –0.20, 0.40, 1.12, 0.19, 1.50 and 1.37 log_{10}/g, respectively.

Karr *et al.* (1996) reported that 97.5 per cent of 40 beef carcasses obtained from two commercial beef slaughter and processing operations had a *Escherichia coli* Biotype 1 count of <10 cfu/cm^2 and for the remaining carcasses, the count was at the level of ≤ 40 cfu /cm^2.

Eisel *et al.* (1997) evaluated the microbiological quality of fresh meat samples taken from different sites of beef carcass, boxed beef, ground beef and retail cuts. *Escherichia coli* count of the samples ranged from 1 to 2 log cfu/g with highest count in ground beef samples.

Gill and Bryant (1997) assessed the hygienic performance of beef carcass cooling processes of plants A and B. They examined 25 beef carcasses, each entering and leaving the chiller. The mean *Escherichia coli* counts of carcasses

that entered and left the chiller in plant A was 0.02 and 0.27 $\log_{10}$ cfu/100 cm^2, respectively. The corresponding counts of carcasses that entered and left the chiller in plant B were 1.08 and –0.26 $\log_{10}$ cfu/100 cm^2.

Linton *et al.* (1997) reported that 67 fresh and frozen beef samples examined by pour plate method had a mean *Escherichia coli* count of 1.67 ± 0.80 log cfu/g and the count of the samples tested by petrifilm method was 2.13 ± 0.78 log cfu/g.

Jericho *et al.* (1998) conducted verification of the hygienic adequacy of cooling process for 120 beef carcass samples taken from lateral rump, sacrum and brisket. The pooled samples collected at the end of slaughter had *Escherichia coli* count of 0.396 ± 0.41 MPN growth units and the corresponding count at the end of cooling process was 0.198 ± 0.058 MPN growth units.

Radu *et al.* (1998) examined 25 beef samples purchased from retail stores in Malaysia. They reported that the presumptive *Escherichia coli* counts of the nine O157:H7 positive samples ranged between 1.2×10^4 and 3.7×10^4 /g.

Sofos *et al.* (1999) reported that in 74.7 to 100.0 per cent of 3,780 beef carcasses collected from seven beef slaughter units had a *Escherichia coli* biotype 1 count of $\leq 10^1$ cfu /cm^2.

Bacon *et al.* (2000) analysed the microbial quality of 1,280 beef carcasses at both hide on and hide off locations. *Escherichia coli* count on the carcasses after hide removal ranged from 2.6 to 5.3 log cfu/100 cm^2.

Gill and Jones (2000) reported that *E. coli* count of 25 dressed beef carcasses each, collected from plants B and C using cotton wool was 1.52 and 2.17 $\log_{10}/cm^2$, respectively. The count of 25 cooled beef carcasses collected from plant D using cotton wool was 0.60 $\log_{10}/125cm^2$.

Jericho *et al.* (2000) analysed microbiological quality of 60 boxed beef cuts collected from high line-speed abattoir and reported that the samples had a mean *E. coli* count of 0.65 $\log_{10}$ most probable number growth units/ cm^2.

Jolly (2000) assessed the bacterial quality of 75 market beef samples collected from four different places in Thrissur. The study revealed that the overall mean *Escherichia coli* count of samples was 3.52 ± 0.09 $\log_{10}$ cfu/g.

Gill *et al.* (2001) compared the efficiency of recovery of bacteria by excision and swabbing with cotton wool, sponge and gauze on randomly selected sites on fat and lean surfaces of 25 beef primal cuts collected from a beef packing plant. The cuts sampled by excision method had a mean *Escherichia coli* count of 2.57 $\log_{10}/100cm^2$ on fat surfaces, but the corresponding counts of samples collected by swabbing with sponge and gauze were 2.39 and 1.5 $\log_{10}/100$ cm^2. The samples obtained from lean surface by excision, swabbing with cotton wool, sponge and gauze had mean *Escherichia coli* counts of 2.42, 1.95, 2.0 and 1.72 $\log_{10}/100$ cm^2, respectively.

Mathew (2001) assessed the microbial quality of 100 beef samples collected from retail meat shops and reported that the overall mean *Escherichia coli* count of the samples was 3.56 ± 0.17 $\log_{10}$ cfu/g.

Martinez *et al.* (2002) examined bacterial load on neck, brisket, and renal sites of 67 bovine carcasses collected from two bovine slaughter houses after skinning, splitting and washing. The mean *Escherichia coli* counts on neck, brisket and renal sites after washing were 1.4, 1.2 and 1.4 log cfu/cm^2, respectively.

Ransom *et al.* (2002) evaluated the efficiency of recovery of *Escherichia coli* Biotype 1 using sponge swabbing, excision, pattern mark swabbing and thorax swabbing on 240 beef carcass samples collected from the five commercial packing plants A, B, C, D and E. The study revealed that the mean log values of *Escherichia coli* Biotype 1 counts recovered by sponge swabbing, excision,

pattern mark swabbing and thorax swabbing method were 1.21, 1.22, 1.03 and 0.99 cfu/ml respectively.

2.1.4. Faecal Streptococcal Count

Kuttinarayanan and Soman (1985) reported that the mean streptococcal count of meat samples collected from slaughterhouse attached to College of Veterinary and Animal Sciences was 28.11 ± 6.67 x 1000/g. The count on the meat samples obtained from meat stall in Panchayat market at Mannuthy was 40.05 ± 3.75 x 100/g. The mean count of the samples collected from East Fort and West Fort meat stalls was 52.6 ± 4.15 x 1000/g and 57.71 ± 4.22 x 1000/g, respectively.

Hudson *et al.* (1986) recorded that the minced beef samples collected from eleven butcher's shops had a mean streptococci count of 1.45 $\log_{10}$/g. The samples taken from seven supermarkets had a mean count of 1.91 $\log_{10}$/g.

Nortje *et al.* (1990) tested the bacteriological quality of retail samples of beef collected before and after sanitation of meat production chain. They reported that the mean enterococci count was 2.4 $\log_{10}$/g, before the start of the day's work.

Latha and Nanu (1994) evaluated the bacteriological quality of beef samples stored at ambient temperatures and recorded a mean faecal streptococcal count of 1.95 ± 0.025 $\log_{10}$ cfu/cm^2 at zero hour of slaughter.

Ziauddin *et al.* (1994) evaluated the bacteriological quality of 12 buffalo carcasses each, obtained from two slaughterhouses. The samples obtained from one of the slaughterhouses had mean enterococcal count of 3.44 $\log_{10}$/cm^2 and the count in the samples belonging to the other slaughterhouse was 2.84 $\log_{10}$/cm^2.

Hinton *et al.* (1998) examined 166 boxes of frozen beef primal cuts samples taken from upper, middle and lower part of the carcasses collected from

44 boning plants during various periods. The study revealed that the mean enterococci count in 12 samples was 0.20 $\log_{10}$ cfu/cm^2.

Jolly (2000) assessed the bacterial quality of market beef samples collected from four different retail market areas. The overall mean faecal streptococcal count obtained from the samples was 4.11 ± 0.07 $\log_{10}$ cfu/g.

Mathew (2001) evaluated the bacteriological quality of 100 market beef samples collected from retail shops in and around Thrissur. The study revealed that the samples had an overall mean faecal streptococcal count of 3.04 ± 0.09 $\log_{10}$ cfu/g.

2.2. ISOLATION AND IDENTIFICATION OF BACTERIA

2.2.1. *Escherichia coli*

Heuvelink *et al.* (1997) examined 1000 raw minced beef samples by plating method, 92 samples by PCR and 201 samples using petrifilm technique, to detect the prevalence of verotoxin (VT) – producing *Escherichia coli* 0157. The organism was not detected in samples of raw minced beef by former method. The organism was isolated from 13 (14.1 per cent) of 92 samples tested by PCR technique and 6 (30 per cent) of 201 samples examined by petrifilm method.

Singh *et al.* (1996) analysed 45 buffalo meat samples collected from retail outlet and reported the isolation of seven faecal *Escherichia coli* from 15.5 per cent of samples.

Cerqueira *et al.* (1997) examined 105 raw beef samples consisting of 35 each of refrigerated ground beef, frozen hamburger patties and frozen meatballs. They isolated 73 diarrhagenic *Escherichia coli* from 34 beef samples and the isolates belonged to 42 strains.

Pierard *et al.* (1997) examined 1,532 beef carcasses and reported the isolation of verocytotoxin producing *Escherichia coli* from 27 (1.8 per cent) samples.

Korsak *et al.* (1998) examined 62 beef carcass samples collected from five beef slaughter houses and reported the isolation of *Escherichia coli* 0157 from three (5 per cent) of these samples.

Radu *et al.* (1998) reported isolation of 12 strains of *Escherichia coli* 0157:H7 from nine out of 25 beef samples purchased from retail stores.

Heuvelink *et al.* (1999) examined raw beef, raw minced beef samples obtained from supermarkets and butchers shops in Netherlands during 1996 and 1997 to detect the presence of 0157/verotoxigenic *Escherichia coli* strains. In 1996, 0157 verotoxigenic *Escherichia coli* strains were isolated from four (1.2 per cent) of 325 samples of raw beef, and four (1.5 per cent) of 264 samples of raw minced beef. In 1997, two (0.4 per cent) of 469 samples of raw beef and two (0.7 per cent) of 307 samples of raw minced beef were found to be positive for 0157/verotoxigenic *Escherichia coli* strain.

Ingham and Schemidt (2000) examined 47 and 32 beef carcasses in study 1 and study 2, respectively. The former study revealed that eight (17.0 per cent) carcasses were contaminated with presumptive *Escherichia coli* and latter study showed that 11 (34.4 per cent) carcasses were contaminated with the organism.

Banerjee *et al.* (2001) examined 32 buffalo meat samples collected from different localities of Bareilly. The study revealed that 14 (43.75 per cent) samples had *Escherichia coli* and four per cent samples had revealed the presence of verotoxigenic *Escherichia coli*. The serotypes comprised of 08, 068, 084 and 088.

Brooks *et al.* (2001) evaluated 91 beef samples collected from supermarkets and butcheries to detect the presence of shiga toxin producing

Escherichia coli. The study revealed the isolation of the organism from 12.1 per cent of samples.

Chinen *et al.* (2001) determined the occurrence of *Escherichia coli* 0157:H7 in 60 ground beef samples collected from retail outlets in Argentina, by selective enrichment culture method and immuno-magnetic separation. They reported that the recovery of the organism by both these methods was comparable and was isolated from 3.8 per cent of the samples.

Guyon *et al.* (2001) isolated one positive sample of *Escherichia coli* 0157:H7 from 255 beef carcasses examined.

Zhao *et al.* (2001) examined 210 beef samples and reported that 40 (19.0 per cent) samples were contaminated with *Escherichia coli.*

Arthur *et al.* (2002) examined 234 pre-eviscerated beef samples and 321 post processed samples. They reported the isolation of 80 (53.9 per cent) non-0157 Shigatoxin producing *Escherichia coli* from the 334 pre-eviscerated carcasses and 27 (8.3 per cent) non-0157 Shigatoxin producing *Escherichia coli* from post processed samples.

Mathew and Nanu (2002) analysed 100 beef samples collected from retail shops and reported that *Escherichia coli* was isolated from 82 per cent of samples. The isolates belonged to 26 serotypes.

Gomathinayagam *et al.* (2002) examined 53 beef samples collected from butcher stalls and reported the isolation of 18 *Escherichia coli* from the samples.

Rozand *et al.* (2002) analysed 3450 minced beef samples obtained from three different French minced beef industries and reported that 175 (5.07 per cent) samples were contaminated with *Escherichia coli* 0157:H7.

Samadpour *et al.* (2002) evaluated the prevalence of Shign Toxin – producing *Escherichia coli* in 296 samples of Seattle ground beef supply. The study revealed that the overall prevalence of the organism was 16.8 per cent.

Jolly and Nanu (2003) analysed 75 market beef samples processed from retail outlets located in and around Thrissur. They reported that 74 (98.67 per cent) samples had *Escherichia coli.* Among the isolates 80.48 per cent belonged to biotype I and were isolated from 52 (69.53 per cent) samples. Of the isolates 7.62 per cent fell into Biotype II, 5.24 per cent were identified as variants of Biotype I and 6.67 per cent as Biotype II.

2.2.2. *Staphylococcus aureus*

Jay (1961) examined various kinds of meat samples collected from 28 retail grocery stores and meat markets and reported the isolation of 18 typeable and 16 untypeable *Staphylococcus aureus* from beefsteaks.

Das Gupta and Chandra Chaudhury (1975) evaluated the presence of coagulase positive staphylococci in beef samples obtained from a slaughterhouse and several meat stalls in Calcutta. They reported the isolation of the organism from 4.3 per cent of samples.

Pandurangarao (1977) examined 66 buffalo meat samples collected from Bareilly for the presence of coagulase positive staphylococci. They reported that 18 (27.27 per cent) of the samples were contaminated with the organism.

Nkanga and Uraih (1981) analysed 24 fresh beef samples each, obtained from traditional markets and supermarkets. They reported that the organism was recovered from cent percent of samples from traditional market and 66.6 per cent of samples from supermarkets.

Nair *et al.* (1983) reported the isolation of *Staphylococcus aureus* from eight out of 77 quarters of buffalo carcass examined.

Bhadekar *et al.* (1986) reported the isolation of *Staphylococcus aureus* from 95 per cent of buffalo meat samples.

Sokari and Anozie (1990) examined 530 meat and related samples obtained from traditional markets in Nigeria. During the investigation *Staphylococcus aureus* was isolated from 449 (84.7 per cent) of the samples, but the organism was isolated from cent percent of the fresh beef samples.

Lefebvre *et al.* (1992) reported that *Staphylococcus aureus* was not isolated from ground beef samples obtained from local supermarkets.

Vorster *et al.* (1994) examined 47 ground beef samples purchased from supermarket outlets and found that 23.4 per cent of samples were contaminated with *S. aureus.*

Bachhil (1998) evaluated the bacterial quality of meat samples consisting 20 each of fresh, frozen and cooked buffalo meat. During the study they observed that 30 per cent of fresh buffalo meat samples were positive for *S. aureus*.

Hansson (2001) detected coagulase positive staphylococci in nine per cent of the beef samples collected from high capacity slaughterhouses and 16.6 per cent of the samples from low capacity slaughterhouses.

Phillips *et al.* (2001) conducted microbiological examination of sponge and excision samples taken from adjacent sites on 670 beef carcasses collected from establishments supplying export and domestic markets. The study revealed that coagulase positive staphylococci were detected on 24.3 per cent of sponged carcass samples and 25.3 per cent of excised samples.

2.2.3. *Salmonella*

Das Gupta (1974) examined 1479 beef samples and reported the isolation of salmonellae from 38 samples. The strains belonged to *Salmonella*

typhimurium, S. typhimurium var *copenphagen, S. newport, S. london, S. dublin, S. anatum, S.stanely, S. butantan, S. virchow* and *S. kiambu.*

Manickam and Victor (1975) tested 67 beef carcasses collected from cattle abattoir in Madras and reported that salmonellae were isolated from four (5.9 per cent) samples. The isolates included *Salmonella dublin* and *S. welteveredan.*

Shoup and Oblinger (1976) reported the isolation of *Salmonella infantis* from one of the 40 ground beef samples examined.

Nair *et al.* (1983) reported the isolation of *Salmonella newport* from two out of 11 quarters of buffalo carcasses obtained from local slaughterhouses.

Scriven and Singh (1986) investigated the microbial quality of retail beef samples and reported that none of the samples were contaminated with salmonella.

Bachhil and Jaiswal (1988) examined 78 fresh meat samples taken from thigh region of buffalo carcasses and reported the isolation of salmonellae from four (5.13per cent) of the samples. The isolates included Salmonella *anatum*, S.*welteveredan, S. typhimurium* and a rough strain.

Paturkar *et al* (1989) reported the isolation of three strains of *Salmonella mbandaka* and the serotype *S. adlaide* from beef samples.

Karr *et al.* (1996) examined 40 samples of beef carcasses and reported that the samples were free of salmonellae.

Korsak *et al.* (1998) examined 310 beef samples collected from five beef slaughterhouses in Belgium and reported that the samples were free from salmonella.

Kimura *et al.* (1999) tested 26 beef samples purchased from local retail meat shops and reported the isolation of salmonella from two samples.

Sofos *et al.* (1999) examined 3,780 beef carcass samples collected from four-steer heifer and three cow bull-slaughtering plants during wet and dry season. They reported that 0.6 per cent of samples were positive for salmonella during wet season and 0.8 per cent of samples were positive for salmonella during dry season in steer heifer slaughtering plants. The organism was isolated from 3.0 and 1.8 per- cent samples during wet and dry season respectively, in cow-bull slaughtering plant.

Agarwal *et al* (1999) reported the isolation of *Salmonella stockholm* from buffalo beef for the first time in India.

Gashe and Mpuchane (2000) reported the isolation of 11 (9.9 per cent) salmonellae from 55 samples of minced meat collected from 12 butcheries situated in different localities on Gaborone.

Phillips *et al.* (2001) examined sponge and excision samples taken from 670 beef carcasses obtained from establishments supplying meat for export and domestic markets. They reported the isolation of salmonellae from 0.2 per cent of sponged samples and 0.6 per cent of excision samples.

Beach *et al.* (2002) examined 100-feedlot cattle carcass and 96 adult cattle carcass samples to detect the presence of salmonella. They reported that 19 per cent of feedlot cattle carcasses were positive for salmonella.

Zhao *et al.* (2002) examined 404 ground beef samples obtained from retail stores and reported that 14 (3.5 per cent) samples were positive for salmonellae. The isolates included serogroups B, C, C2, and E and the serotypes were *Salmonella typhimurium* var *copenphagen, S. meliagridis, S. kentucky, S. muensten* and *S. cerro*.

Ransom *et al.* (2002) isolated salmonella from two (6.7 per cent) out of 30 beef carcasses.

2.2.4. *Listeria monocytogenes*

Prasai *et al.* (1991) evaluated the microbial effect of hot lactic acid sprayed on 40 beef carcasses before chilling. They reported that listeria was detected in three untreated samples.

Wang *et al.* (1992) examined 10 beef samples and reported isolation of *Listeria spp.* other than *L. monocytogenes* from 70 per cent of the beef samples.

Karr *et al.* (1996) reported the isolation *Listeria monocytogenes* from one out of 40 beef carcasses examined.

Ryser *et al.* (1996) determined the incidence of *Listeria spp.* in 45- retail raw ground beef samples and reported that 40 (89 per cent) out of 45 samples had listeria. The species isolated consisted of *L. monocytogenes, L. innocua* and *L. welshimeri.*

Duffy *et al.* (1997) tested minced beef samples obtained from local butcher shops and supermarkets in Netherlands and reported the isolation of *L. monocytogenes, L. innocua* from four samples each and *L. welshimeri* from two samples.

Prasai *et al.* (1997) isolated *Listeria spp.* from 28 per cent of untreated beef loin samples and four per cent of 1.5 per cent lactic acid spray-treated loins.

Korsak *et al.* (1998) tested 200 beef carcasses collected from five cattle slaughter houses in Belgium and reported the isolation of *L. monocytogenes* from 50 (22 per cent) beef carcasses.

Baek *et al.* (2000) examined the incidence of *L. monocytogenes* in 70 raw beef samples collected from randomly selected supermarkets and quarantine

stations. In this study *L. monocytogenes* was isolated from 3 (4.3 per cent) beef samples.

2.3. BACTERIAL COUNT ON THE SAMPLES OF ENVIRONMENT AND PROCESSING EQUIPMENT

Rao and Ramesh (1992) analysed water, knives, floors, wall, processing equipment and hand wash of workers before and after slaughtering operation, to identify the critical points of microbial contamination in the slaughter line. The study indicated that the total viable count of the samples before slaughtering operation was at the level of 10^2 cfu/cm^2 or per ml in floor washings, wall and equipment. The mean count in the water and knives was at the level of 10 cfu/ cm^2 or per ml. They observed a four fold increase of the count in the samples collected after slaughter.

Tarwate *et al.* (1993) evaluated the potential source of microbiological hazard associated with slaughter line operations and the surrounding operations. They examined knife, axe, saw blade, hooks, floor, wall, platform, hand swab and water and reported that the total viable count of these samples varied from 6.70 $\log_{10}$ cfu/cm^2 to 2.9 $\log_{10}$ cfu/cm^2. The coliform count ranged between 6.9 $\log_{10}$ cfu/cm^2 and 4.2 $\log_{10}$ cfu/cm^2. Water samples revealed the lowest total viable count and the mean count of the sample was 2.07 $\log_{10}$ cfu/ml. During the study they isolated 651 bacterial organisms, which included potential pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium spp*, *Escherichia coli* and *Shigella spp*, and also spoilage organisms.

Karr *et al.* (1996) estimated the bacterial count of 20 cutting tables, conveyor belt surface samples and reported that aerobic plate count on the conveyor surfaces were lower with all samples under 10^3 cfu/cm^2 and 53.7 per cent under 50 cfu/ cm^2. The total coliform counts from cutting tables and conveyor belts was 10^2 cfu/ cm^2 and 44 per cent had counts of $<$ one cfu/ cm^2.

For counts of *Escherichia coli* Biotype 1, 51 per cent of conveyor surfaces samples had counts of <10 cfu/cm^2 and 46 per cent had counts of < one cfu/cm^2.

Eisel *et al.* (1997) evaluated the microbial load of air, floor and processing equipment in a red-meat processing plant. Sanitized processing equipment had a mean total viable count of one cfu/cm^2 and the count in the floor was 5 cfu/cm^2 Coliforms and *Escherichia coli* were rarely recovered from these samples. The total viable count of air samples was generally low (0.6 cfu/m$^{2)}$ and the count in the carcass receiving area was 2.4 cfu/m$^{3.}$

Rahkio and Korkeala (1997) studied the microbiological contamination of air in four slaughterhouses by using impactor samples taken from back splitting and weighing areas. The mean aerobic count of the air in the former area was 2.25 $\log_{10}$ cfu/100 l of air and the count in the latter area was 2.03 $\log_{10}$ cfu/100 l of air.

Gill *et al.* (1999) analysed the samples collected from cleaned processing equipment and steel mesh gloves of workers in a sheep carcass breaking process. They reported that the samples from conveyor belt support bars had a mean total aerobic count of 2 to 5 $\log_{10}$ cfu/cm^2 and the count one steel mesh gloves was 7 to 9 $\log_{10}$ cfu/cm^2. The coliform and *Escherichia coli* counts on steel mesh gloves were greater than 3.0 $\log_{10}$ cfu/cm^2.

2.4. BACTERIOLOGICAL STANDARDS

Karr *et al.* (1996) cited that 93.1 per cent of carcass samples had total viable count under 10^4 cfu/cm^2 according to the Food Safety and Inspection Service (FSIS) microbiological study.

The government of India has prescribed microbiological standards for chilled/frozen buffalo meat, veal, mutton and minced meat (Rao *et al.*, 1998). The standards recommend that three of the five samples tested should not have a

total plate count more than 10^6/g and in remaining two samples the count should not exceed 10^7/g.

The international commission of microbiological specification for foods recommended that the total viable count at 35°C should be less than 10^7/g (Gracey *et al.*, 1999).

Karr *et al.* (1996) cited that according to FSIS microbiological study the total coliform count in 96.4 per cent carcasses were less than 10^2 cfu/cm^2.

According to agricultural produce (Grading and Marketing) (1979), Government of India the raw chilled frozen buffalo meat should not have *Escherichia coli* count exceeding 10/g.

According to FSIS microbiological study, 97.5 per cent carcasses had *Escherichia coli* Biotype 1 count at the level of $\leq$ 10 cfu/cm^2 (Karr *et al.*, 1996).

The Government of India standards for raw meat (chilled/frozen) prescribed that salmonella should be absent in all the five samples examined (Rao *et al.*, 1998).

The zero tolerance policy of FDA on listeria advocated the absence of *Listeria monocytogenes* in 50 g of food. The policy also prescribed that if food contains more than 10^4 *Listeria monocytogenes* per gram, a recall is automatic (Farber, 1993).

Materials and Methods

3. MATERIALS AND METHODS

During the investigation a total of 40 beef carcasses were randomly selected from a meat processing plant located at Kochi in Kerala. The factory procures the carcasses from two sources (A and B) located at Tamil Nadu for fabrication and production of meat and its products.

3.1. COLLECTION OF SAMPLES

In the present investigation 20 beef carcasses each were randomly selected from the sources A and B. In each visit only two carcasses from one source was selected for sampling. In order to represent each carcass, five sites viz., neck, brisket, loin, flank and outer round were sampled and is shown in plate 1.

3.1.1 Sampling Technique

An area of 100 cm^2 was marked using a sterile aluminum template of 10 x 10 cm size at each swabbing area of the carcass sites. This selected area was swabbed with a sterile cotton swab of about 2.5 cm breadth. Before swabbing, the cotton swabs were hydrated in 0.1% peptone water and the excess diluent in the swab was removed by gentle pressing of the swab on the inside of flask containing 100 ml sterile diluent. Each marked area of a carcass was swabbed lengthwise and breadthwise using the sides of the swab and from corner to corner with the tip of the swab and transferred in to the flask containing 100 ml diluent. The samples were brought to the laboratory in a thermocool container. All aseptic precautions were taken during the sample collection, processing, estimation of bacterial load and isolation and identification of bacteria.

3.2. PROCESSING OF SAMPLES

All five swab samples collected from each carcass were cut with a pair of sterile scissors and dropped in to the flask containing diluent. The contents of the

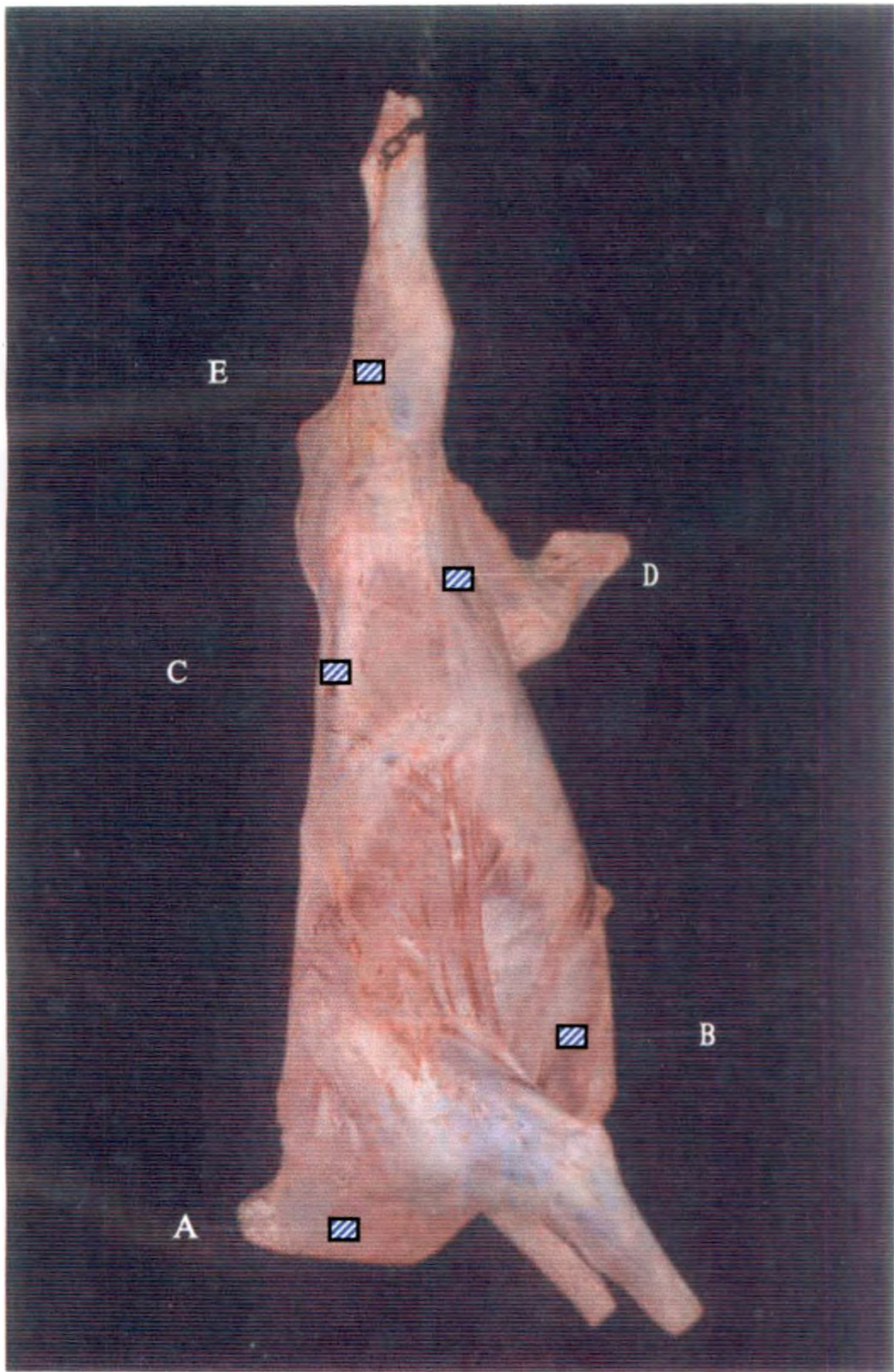


Plate 1. Sampling sites on beef carcass:
(A) Neck (B) Brisket (C) Loin (D) Flank (E) Outer round

flask was thoroughly agitated with the help of a cyclomixer at 8000 rpm for 3 min so as to extricate the bacteria attached to the cotton swab in to the diluent. The agitated five swab samples collected from a carcass were pooled and this formed the initial test sample of a carcass.

3.3. PREPARATION OF SAMPLES

In order to estimate the bacterial load per cm^2 of the carcasses, 25 ml of the initial test sample was transferred in to 225 ml of the diluent so as to form 1 in 10 dilution of the sample. Transferring one ml of inoculum in to 9.0 ml of the diluent made further ten fold dilutions. Serial dilutions of each sample were made up to 10^{-9} (Swanson *et al.,* 2001). The selected serial dilutions of each sample were used to estimate the level of various bacteria per cm^2.

3.4. BACTERIAL COUNT

The initial test sample prepared from each carcass was tested to estimate the total viable count (TVC), Coliform count (CC), *Escherichia coli* count (ECC) and Faecel streptococcal count (FSC).

3.4.1. Total Viable Count

Total viable count (TVC) of each sample was estimated by pour plate technique, as described by Swanson *et al.* (2001). From the selected ten fold dilution of each sample, one ml of the inoculum was transferred on to duplicate petridishes of uniform size. To each of the inoculated plates about 15-20 ml sterile molten standard plate count agar (Hi-media) maintained at 45°C was poured and mixed with the inoculum, by gentle rotatory movement i.e., clock wise, antilock wise, forward and backward. The inoculated plates were left at room temperature and allowed to solidify, and incubated at 37°C for 24 h. At the end of incubation, plates showing between 30 and 300 colonies were selected and counts were taken with the help of a colony counter. The number of colony

forming units (cfu) per cm^2 of carcasses was calculated by multiplying the mean colony count in duplicate plates with the dilution factor and expressed as cfu/cm^2.

3.4.2. Coliform count

Coliform count per cm^2 of carcasses was estimated according to the procedure described by Nordic Committee (1973). From the selected dilution, 0.1 ml of the inoculum was inoculated on to duplicate plates of violet red bile agar (VRBA) (Hi-media) and was uniformly distributed with a sterile 'L' shaped glass rod. The plates were incubated at 37°C for 24 h. At the end of incubation, purplish red colonies with diameter of at least 0.5 mm, surrounded by a reddish zone of precipitate were counted as coliforms. The number of organisms per cm^2 of carcasses were estimated as described in total viable count and expressed as cfu/cm^2.

3.4.3. *Escherichia coli* Count

The number of *Escherichia coli* per cm^2 of carcasses was estimated as prescribed by Indian standards (1980). To estimate the organisms, 0.1 ml of inoculum from the selected dilution was transferred on to duplicate plates of Eosin Methylene Blue (EMB) agar (Hi-media) and was evenly distributed over the medium with a sterile 'L' shaped glass rod. The plates were incubated at 37°C for 24 h. After the incubation period, colonies with a greenish black metallic sheen on deflected light were counted as *Escherichia coli*. The number of organism per cm^2 of carcasses were estimated as described in total viable count and expressed as cfu/cm^2.

3.4.4. Faecal Streptococcal Count

The standard procedure prescribed by Nordic Committee (1968) was followed to estimate the number of faecal streptococci per cm^2 of carcasses. Accordingly, 0.1 ml of the inoculum from the selected dilution was transferred on to duplicate plates of Karl Friedrich (KF) streptococcal agar (Hi-media). The

inoculum was uniformly distributed on to the plates using an 'L' shaped glass rod. The plates were incubated at 37°C for 48 h. Pink to dark red colonies with a diameter between 0.5 and three mm and surrounded with a narrow whitish zone were counted as faecal streptococci. The number of organisms per cm^2 of carcasses were estimated as described in total viable count and expressed as cfu/cm^2.

3.5. ISOLATION AND IDENTIFICATION OF BACTERIA

Each carcass swab sample was used for the isolation and identification of *Escherichia coli, Staphylococcus aureus,* Salmonellae and *Listeria monocytogenes.*

3.5.1. *Escherichia coli*

A loopful of inoculum from the initial test sample was inoculated on to duplicate plates of EMB agar and incubated at 37°C for 24 h. At the end of incubation, three or four colonies with characteristics of *Escherichia coli* were transferred on to nutrient agar slants and incubated at 37°C for over night. These isolates were tested for further characterization and identification by cultural, morphological and biochemical reactions described by Barrow and Feltham (1993) and are shown in flow chart 1. *Escherichia coli* isolates were subjected to Eijkman test and were serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh.

3.5.2. *Staphylococcus aureus*

For the isolation of *Staphylococcus aureus*, a loopful of the initial test sample was inoculated on to Baird-Parker (BP) agar medium (Hi-media) and was incubated at 37°C for 48 h (Lancette and Bennett, 2001). At the end of incubation, colonies showing characteristic appearance (circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray black to jet black, frequently with light coloured margin, surrounded by opaque zone and frequently

Flowchart 1. Isolation and identification of *Escherichia coli*

Step	Characteristics/Reactions
Initial test sample solution	
↓	
Inoculated in EMB agar (in duplicate plates)	
↓	
Suspected colonies (colonies with dark center with distinct indelible ink, greenish black metallic sheen on deflected light)	
↓	
Nutrient agar	
↓	
Grams' staining reaction and cell morphology	Gram negative small rods
↓	
Motility test	+
↓	
Growth aerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	F
↓	
Urease	-
↓	
ONPG	-
↓	
Indole	+
↓	
MR	+
↓	
VP	-
↓	
Citrate Utilization test	-
↓	
Carbohydrate utilization	
Lactose	+
Gluose	+
Mannitol	+
Inositol	-
Maltose	+

F= fermentation, + = positive, - =negative

with outer clear zone) on BP agar medium were selected and transferred to nutrient agar slants and incubated at 37°C for overnight. The isolates were stored at refrigeration temperature. Characterization and identification of the isolates were done following the procedure described by Barrow and Feltham (1993) and are show in the flow chart 2. The isolates were identified based on the cultural, morphological and biochemical characteristics.

3.5.3. Salmonellae

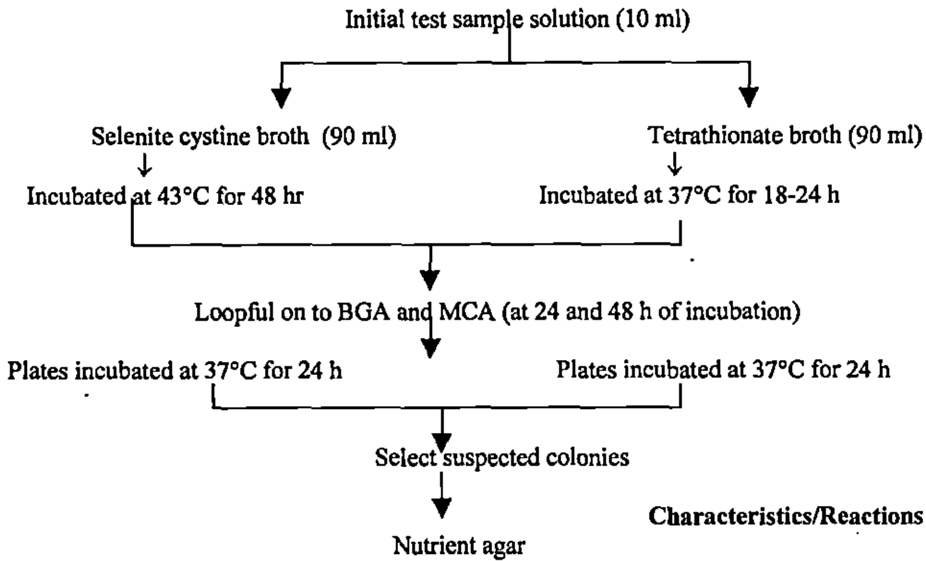
In order to isolate salmonellae from each carcass sample, 10 ml each of initial test sample solution was transferred in to sterile conical flask containing 100 ml of tetrathionate broth (Hi-media) and an equal quantity of selenite cystine broth (Hi-media) (Cox *et al.*, 1981). The contents of the flask were mixed thoroughly and the flask containing tetrathionate broth was incubated at 37°C for 48 h and the flask containing selenite cystine broth was incubated in a water bath at 43°C for 48 h. At the end of 18 and 24 h of incubation, a loopful of the culture from each of tetrathionate broth and selenite cystine broth was inoculated on to each of duplicate plates of MacConkey Agar (MCA) and Brilliant Green Agar (BGA) (Hi-media) and incubated at 37°C for 24 h. At the end of incubation, translucent, colourless colonies with opaque center (Andrews *et al.*, 2001) were selected from MCA plates and colourless pink white opaque to translucent colonies with a diameter of about 1-2 mm, surrounded by a pink or red hue were selected from BGA plates. The selected colonies were transferred to nutrient agar slants and incubated at 37°C for overnight and stored at refrigeration temperature for further characterization of the isolates. The cultural, morphological and biochemical characteristics of the isolates were identified according to the procedure described by Edwards and Ewing (1972) and Barrow and Feltham (1993) and are shown in flow chart 3.

Flow chart 2. Isolation and identification of *Staphylococcus aureus*

	Characteristics/Reactions
Initial test sample solution	
↓	
Inoculated on to BP agar (in duplicate plates)	
↓	
Select suspected colonies (circular, smooth, convex, moist, 2-3 mm in diameter, gray – black to jet – black frequently with light coloured margin, surrounded by opaque zone and frequently with outer clear zone; buttery to gummy consistency)	
↓	
Nutrient agar	
↓	
Gram's staining reaction and cell morphology	Gram positive cocci in singles, pairs, cluster or bunch of grapes appearance
↓	
Motility test	-
↓	
Growth aerobically	+
↓	
Growth anaerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	F
↓	
VP	+
↓	
Arginine hydrolysis	+
↓	
Phosphatase	+
↓	
Gelatin liquefaction	+
↓	
Urease	+
↓	
Coagulase test	+
↓	
Carbohydrate utilization	
Glucose	+
Lactose	+
Mannitol	
Aerobic	+
Anaerobic	+

F = fermentation, + = positive, - = negative

Flow chart 3. Isolation and Identification of *Salmonella*



Test	Characteristics/Reactions
↓ Gram's reaction and cell morphology	Gram negative small rods
↓ Motility	+
↓ Growth aerobically	+
↓ Catalase	+
↓ Oxidase	-
↓ OF test	F
↓ Urease	-
↓ TSI	Alkaline slant, acid butt, with or without H_2S and gas
↓ Lysine decarboxylase test	+
↓ Arginine hydrolysis test	+
↓ Phenyl alaninedeaminase test	-
↓ Indole	-
↓ MR	+
↓ VP	-
↓ Citrate utilization	+
↓ Carbohydrate utilization mannitol	+
Lactose	-
salicin	-

F=fermentation + = positive, - = negative

3.5.4. Listeria monocytogenes

The procedure used for the isolation and identification of *L. monocytogenes* was similar to that described by Wang *et al.* (1992). Each carcass swab sample (10 ml) was mixed with 90 ml of Listeria Enrichment Broth (LEB, Hi-media) and incubated at 30°C for seven days. After second and seventh days of enrichment, samples were streaked on to Oxford and Polymixin B Acriflavin Lithium chloride Ceftazidime Aesculin Mannitol (PALCAM) agar (Hi-media) plates. The plates were incubated at 30°C for 28-48 h, and then five typical aesculin positive colonies per plate were streaked on to Lithium Phenylethanolchloride Moxalactum (LPM) agar (Hi-media) and incubated at 30°C for 24 h. At the end of incubation, plates were observed under Henry's oblique lighting technique. Grayish blue typical bacterial colonies were picked, streaked on to Trypticase Soy Agar (TSA) slants, incubated at 30°C for 24 h and stored at refrigeration temperature for further characterization of the isolates. The isolates were subjected to various tests as shown in the flow chart 4.

3.6. CHARACTERIZATION AND IDENTIFICATION OF ISOLATES

The suspected colonies selected as *Escherichia coli, Staphylococcus aureus,* salmonellae and *Listeria monocytogenes* were subjected to various tests and identified based on the cultural, morphological and biochemical characteristics described by Barrow and Feltham (1993), except for the triple sugar iron agar test (Edwards and Ewing, 1972).

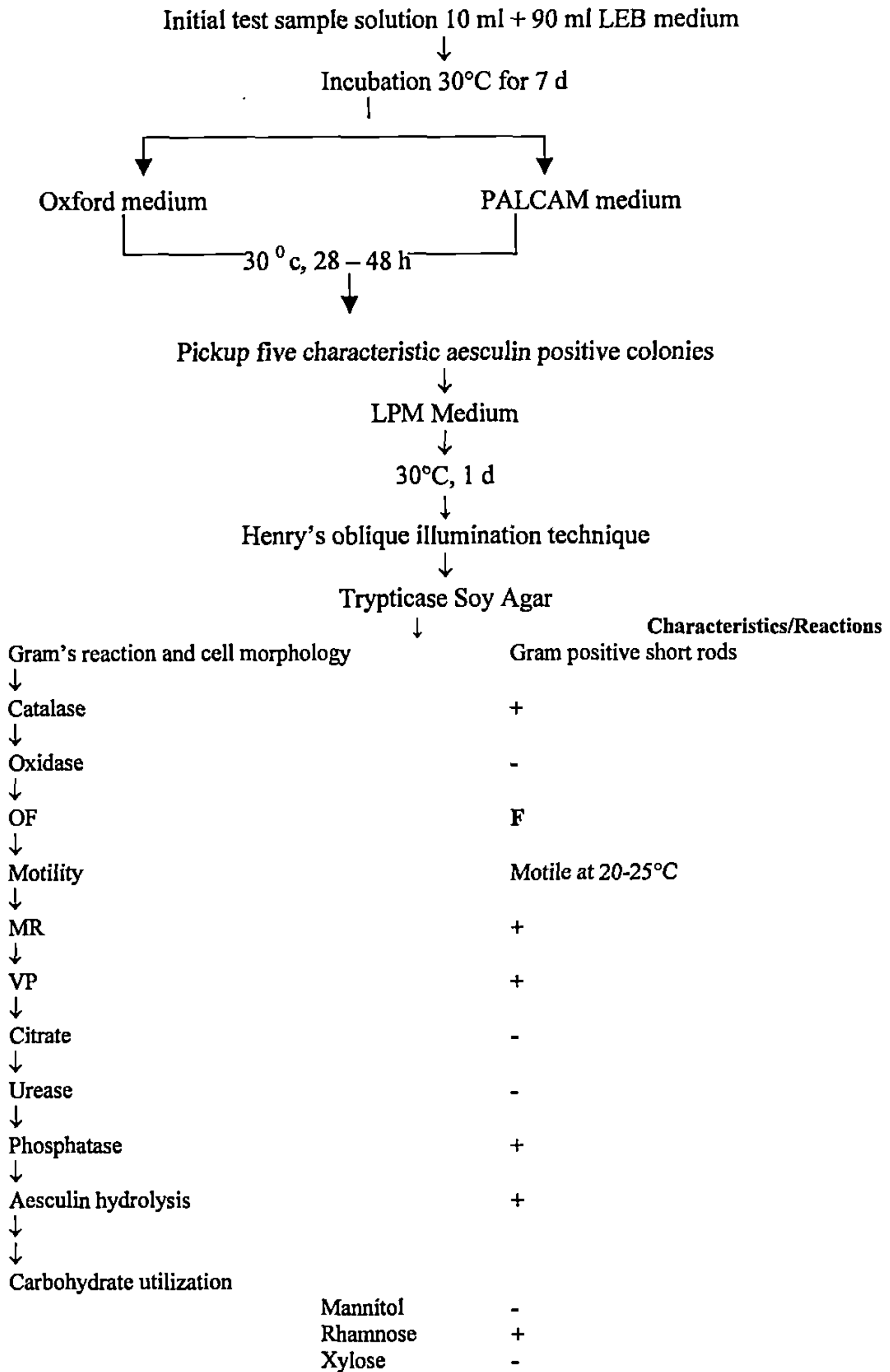
3.6.1. Primary Identification Test

Catalase test

a) Slide test

A small quantity of colony was transferred on to a clear, grease free, glass slide and mixed well with a drop of three per cent hydrogen peroxide. Evaluation of effervescence with in a few seconds indicates a positive reaction.

Flowchart 4. Isolation and identification of *Listeria monocytogenes*



F =fermentation, + = positive, - = negative

b) Tube test

One ml of three percent hydrogen peroxide solution was poured over the slope of a nutrient agar slant on which the isolate was grown. A positive reaction is indicated by the development of effervescence immediately.

Gram staining

The procedure for gram staining is as follows:

a. A thin smear of each isolate was made on a clean, grease free glass slide. Air-dried the smear and then heat fixed by passing over a flame.

b. The smear was then flooded with 0.5 per cent crystal violet in water and allowed to act for 30 seconds.

c. Poured off the stain and washed with water.

d. Flooded the smear with Gram's iodine solution (one per cent iodine and two per cent potassium iodide in water) for 30 seconds.

e. Poured off the solution and the smear was decolourised with a few drops of acetone and allowed to act for two to three seconds.

f. Washed the smear and counter stained with dilute carbol fuschin for 30 seconds.

g. Poured off the stain from the slide, washed, dried and examined under oil immersion objective of the microscope.

Motility test

Motility of the organism was assessed by stabbing the isolate in to the Hugh and Leifson's medium with a straight wire up to a depth of 5 mm. Motility is indicated by a spreading growth in to the medium from the line of inoculations and growth of non-motile organisms is confined to the stab.

Oxidase test

A filter paper strip is moistened with a few drops of an aqueous solution of 1 per cent tetramethyl paraphenyline diamine dihydrochloride. Each isolate was then smeared across the paper strip with a platinum loop. The appearance of a dark purple colour on the paper strip within 30 seconds indicates a positive reaction.

Oxidation – Fermentation test

Each isolate was inoculated in to duplicate tubes of Hugh and Liefson's media by stabbing with a straight wire. One of the tubes was sealed with a layer of melted soft paraffin to a depth of about 3 cm above the medium. The tubes were incubated at 37°C for up to 14 days. A change in colour of the medium from green to yellow in the open tubes alone is taken as oxidation whereas a change in colour from green to yellow in both the tubes is regarded as fermentation. Absence of colour change in both tubes indicates no action on carbohydrates.

3.6.2. Secondary Tests

Aesculin hydrolysis

The organism was inoculated in to aesculin broth and was incubated at 37°C and examined daily for five days. Blackening of the broth due to hydrolysis of aesculin indicates a positive reaction.

Arginine hydrolysis

The organism was inoculated in to five ml of arginine broth and was incubated at 37°C for 24 h. At the end of incubation period, added 0.25 ml of Nessler's reagent. Arginine hydrolysis is indicated by the development of brown colour.

Carbohydrate utilization test

Each isolate was inoculated in to two test tubes containing peptone water with Andrade's indicator and one per cent of the appropriate sugar. One of the tubes contained an inverted Durham's tube. The inoculated tubes were incubated at 37°C and examined daily for seven days to detect the production of acid and/or gas. A change in colour of the medium to pink indicates acid production and the production of gas is indicated by the appearance of air bubbles in the inverted Durham's tube. Anaerobic condition of the medium was provided by adding a layer of sterile molten soft paraffin to a depth of about one centimeter above the media.

Citrate utilisation test

A light suspension of the organism was made in normal saline and was inoculated with a straight wire on to the slope of Simmon's citrate agar. The inoculated medium was incubated at 37°C and examined daily up to seven days. The ability of the organism to utilize citrate as the sole source of carbon is indicated by a change in colour of the medium from green to blue and growth of the organism along the streak line.

Coagulase test

a) Slide test

A small quantity of the culture was emulsified in a drop of saline on a microscope slide to produce a thick suspension. The suspension was stirred with a straight wire dipped in rabbit plasma. Macroscopic clumping with in few seconds indicates a positive result and delayed clumping is considered as a negative reaction.

b) Tube test

Mixed 0.5 ml of undiluted rabbit plasma with an equal volume of an 18 to 24 h broth culture of the test organism and incubated at 37°C and examined after one and four hours for coagulation. Negative tubes were left at room temperature overnight and re-examined.

Decarboxylase reaction

Each isolate was heavily inoculated with straight wire in to three test tubes containing decarboxylase media. One of the tubes contained lysine and other contained ornithine. The third tube was taken as the control. The organism was inoculated through the paraffin layer and incubated at 37°C for five days. In a positive reaction, the medium first turns yellow and then becomes purple and the control tubes remain yellow.

Eijkman test

Each test organism was inoculated in to tubes containing MacConkey broth with inverted Durham's tube, warmed to 37°C and incubated at 44 ± 0.1°C in a water bath for 48 h. Production of both acid and gas indicates a positive reaction.

Gelatin hydrolysis/liquefactions

Each isolate was inoculated in to nutrient gelatin and incubated at 37°C up to 14 days. An uninoculated control tube was also set. The tubes were cooled every two to three days in a refrigerator for 2 h and then examined for liquefaction. A positive result is indicated by liquefaction of gelatin.

Hippurate hydrolysis

The slope of hippurate agar was lightly inoculated with the test organism and examined daily for seven days. Hydrolysis of hippurate is indicated by growth and the development of a pink colour due to alkali production.

Indole production

The isolate was inoculated in to peptone water and incubated at 37°C for 48h. At the end of incubation added 0.5 ml of Kovac's reagent, mixed well and examined. A red colour in the reagent layer indicates a positive reaction.

Methyl red (MR) reaction

The MR-VP medium was inoculated with the isolate and incubated at 37°C for two days. Added two drops of methyl red solution at the end of incubation period and examined. Development of a red colour indicates positive reaction.

ONPG (O-nitrophenyl-P-D-glactopyranocide) test

Each isolate was inoculated in to ONPG broth and incubated at 37°C for 48h. The p-galactosidase activity of the organism was indicated by the development of a yellow colour due to the production of O-nitrophenol.

Phenylalanine deamination

The phenylalanine agar slope was heavily inoculated with the test organism and incubated at 37°C for overnight. At the end of incubation, 0.2 ml of 10 per cent aqueous solution of ferric chloride was poured over the slope. A positive result is indicated by the development of a green colour on the slope and in the free liquid at the base.

Phosphatase test

The phenolphthalene phosphate agar was lightly inoculated with the test organism to obtain discrete colonies and incubated at 37°C for 18 h. At the end of incubation, 0.1 ml of ammonia solution (specific gravity –0.880) was placed in the lid of the petri dish and the medium was inverted above it. Free phenolphthalein liberated by phosphatase reacts with the ammonia and phosphatase positive colonies become bright pink.

Triple sugar iron agar test

Each isolate was stab inoculated in to the butt of triple sugar iron agar with straight wire and the slope of the agar was streaked with the wire. The inoculated tubes were incubated at 37°C for 24 h. The tubes were examined at the end of incubation for the development of an alkaline slant and an acid butt with or without the production of hydrogen sulphide (Edwards and Ewing, 1972).

1) Urease activity

Slope of Christensen's urea agar was heavily inoculated with the test organism and incubated at 37°C. The tubes were examined after 4 h of incubation and daily for 5 days. Development of a red colour in the medium indicated a positive reaction.

2) Voges-Proskauer reaction

The MR-VP medium inoculated with the isolate was subjected to methyl-red test. After completion of the test, added 0.6 ml of 5 per cent á-naphthol solution and 0.2 ml of 40 per cent aqueous potassium hydroxide in to the tube. After thorough mixing of the contents, the tube was kept in a slanting position and examined after 15 min and one hour. A positive reaction is indicated by the development of a strong red colour.

3.7. ENUMERATION OF BACTERIA FROM THE ENVIRONMENTAL SAMPLES, PROCESSING EQUIPMENT AND HAND WASHING OF PERSONNEL

3.7.1. Air

Direct exposure method prescribed by Evancho *et al.* (2001) was employed for the estimation of bacterial counts in the air samples of the slaughter hall and chilling room. To estimate the count duplicate petridishes containing sterile nutrient agar medium were exposed for 15 minutes. The plates were

brought to the laboratory in thermocool container and incubated at 37°C for two days. The number of colonies developed in the duplicate plates was counted and the mean count of the plates was expressed as cfu/min. Examination of air samples were repeated in six visits.

3.7.2. Water

3.7.2.1. Collection of water samples

a) Pond water

Pond water for bacteriological examination was collected following the procedures described by Indian Standards (1978). A clean sterile bottle of 250 ml capacity was held by its bottom and plunged its neck downwards below the surface of the water. The bottle was then turned until the neck pointed slightly upwards. When the bottle was filled with water, it was raised above the surface of water and the stopper was replaced. The water samples were transported to the laboratory in a thermocool container.

b) Tap water

Tap water for bacteriological examination was collected following the procedures described by Indian Standards (1978). Allowed the water from the tap to run to waste for about two minutes in order to flush the interior of the nozzle and discharge the stagnant water. A sterile bottle of 250 ml capacity was used to collect the water. The bottle was held near the base with one hand and filled from a gentle stream of water from the tap, avoiding splashing and brought to the laboratory in a thermocool container.

c) Ice

Procedure for collection of samples was similar to the collection of water samples (Indian Standards, 1975).

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3.7.2.2. Processing of water samples

Water samples brought to the laboratory were shaken vigorously for about 25 times. Removed the stopper and flamed the mouth of the bottle. This formed the initial test sample.

3.7.2.3. Preparation of water sample

In order to estimate the bacterial load per ml of water sample, 10 ml was transferred to 90 ml of sterile quarter strength Ringers solution so as to form one in 10 dilution of the sample. Transferring one ml of inoculum in to nine ml of the diluent formed further 10-fold dilution. Serial dilutions of each sample were made up to 10^7. The selected serial dilution of each sample of water was used to estimate the total viable count (TVC), Coliform count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC) and was carried out as in the case of carcass swab samples. The count obtained was expressed as cfu/ml. The examination of water samples and ice samples was repeated during six visits.

3.7.3. Processing equipments

Swab contact method described by Evancho *et al.* (2001) was followed to collect samples from the meat cutting board, meat cutting tables, knife and steel for the estimation of bacterial count.

a. Cutting board and table

To collect samples from the meat cutting board and meat cutting table a sterile cotton swab was moistened with 0.1 per cent peptone water and excess diluent was removed by pressing the swab against the interior wall of the vial with a rotating motion. The swab head was rubbed slowly and thoroughly over 100-cm^2 surfaces, which was marked, with a sterile aluminium template (10 x 10 cm^2interval area). The swab was rubbed three times reversing the direction between strokes. After swabbing the head of swab was cut with a pair of sterile

scissors, transferred in to sterile flask containing 100 ml of 0.1 per cent peptone water and brought to the laboratory in a thermocool container.

b. Knife and steel

Knife and steel samples were collected with sterile cotton swab moistened as described in the sample collection of cutting board and table. The swab was slowly moved firmly over the entire surface, reversing the direction each time. After swabbing, the transportation of the swab was carried out as described in the sampling of cutting board and table.

c. Hand washings

The samples for the estimation of bacterial load on the hands of personnel, were collected from one randomly selected individual involved in processing operations. The plant was visited six times. On each visit individual's hand was washed in 100 ml of 0.1 per cent sterile peptone water collected in sterile conical flask and brought to the laboratory in a thermocool container.

Examination of cutting board, cutting table, knife and steel was repeated at six visits. The preparation, processing and estimation of bacterial load on the swab samples collected from cutting board, cutting table, knife and steel were carried out as in the case of carcass swab sample.

The bacterial count of the cutting board, and cutting table was expressed as colony forming units (cfu) per cm^2 and knife and steel was expressed as cfu/ml. Hand washes of the personnel were mixed as described by Indian Standards (1978) and further ten fold serial dilutions of samples were made as prescribe din the Indian Standards (1978). The bacterial count of the samples was estimated as described in the carcass swab sample examination and the count was expressed as cfu/ml.

The data obtained in the study were subjected to statistical analysis as per the procedure described by Rangaswamy (1995).

Results

4. RESULTS

A total of 40 randomly selected beef carcasses were sampled from a meat processing plant and examined for total viable count (TVC), coliform count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC). The samples were also subjected to the isolation and identification of *Salmonella, Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes*. The samples of air, water, equipment and hand wash of personnel were also collected and estimated the various bacterial loads of these samples.

4.1. BACTERIAL COUNTS

4.1.1. Total Viable Count

The mean total viable count of carcasses belonging to the sources A and B and also the overall mean count of the samples is given in table 1. Analysis of variance test of the data did not reveal significant difference between the mean count of samples from the two sources. The samples had an overall mean count of 7.40 ± 0.17 $\log_{10}$ cfu/cm^2. The mean count of samples from the sources A and B were 7.32 ± 0.17 and 7.47 ± 0.18 $\log_{10}$ cfu/cm^2, respectively.

Table 1. Mean total viable count of 40 beef samples belonging to Source A and B

Source of carcass	No. of samples tested	Mean ± SE ($\log_{10}$cfu/cm^2)
A	20	7.32 ± 0.17
B	20	7.47 ± 0.18
Overall mean	40	7.40 ± 0.17

Distribution of carcasses based on total viable count

The distribution of carcasses based on total viable count is shown in table 2 and illustrated in fig 1. Of the 40 samples examined, one sample of source A

had a count at the level of 10^9 cfu/cm^2. Only 35 per cent of the carcasses of source A had count at the level of 10^6 cfu/cm^2, whereas, 55 per cent of the carcasses from source B had the count at the level of 10^6 cfu/cm^2. Of the 40 samples examined 45 per cent had count at the level of 10^6 cfu/cm^2. The counts in 30 per cent and 22.5 per cent samples were at the level of 10^8 cfu/cm^2 and 10^7 cfu/cm^2, respectively.

Table 2. Distribution of carcasses based on total viable count

Source of samples	Total viable count on carcass (cfu/cm^2)			
	10^6	10^7	10^8	10^9
A	7 (35)	6 (30)	6 (30)	1 (5)
B	11 (55)	3 (15)	6 (30)	

Figures in the parenthesis indicate percent

4.1.2. Coliform count

The mean coliform count of carcasses belonging to the sources A and B is given in table 3. The analysis of variance test of the data revealed significant difference ($P<0.05$) in the mean count of the samples from the two sources. The samples had an overall mean coliform count of 3.41 ± 0.13 $\log_{10}$ cfu/cm^2.

Table 3. Mean coliform count of 40 beef samples belonging to Source A and B

Source of carcass	No. of samples tested	Mean± SE ($\log_{10}$cfu/ cm^2)
A	20	$3.61^{a*} \pm 0.13$
B	20	$3.21^{a} \pm 0.13$
Overall mean	40	3.41 ± 0.13

Figures bearing the same superscript differ significantly
* $P<0.05$

Distribution of carcasses based on coliform count

The distribution of carcasses based on coliform count is shown in table 4 and illustrated in fig 2. Coliform count of the samples from both the sources varied from 10^2 to 10^4 cfu/cm^2. The count in 55 per cent of the samples from the

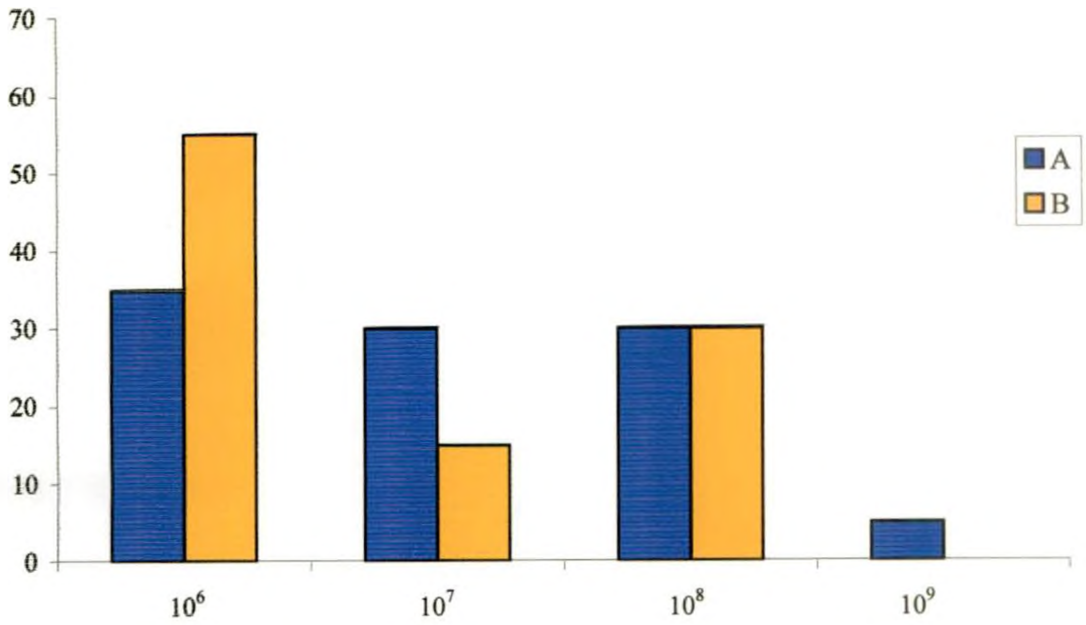

Fig. 1. Distribution of carcasses based on total viable count

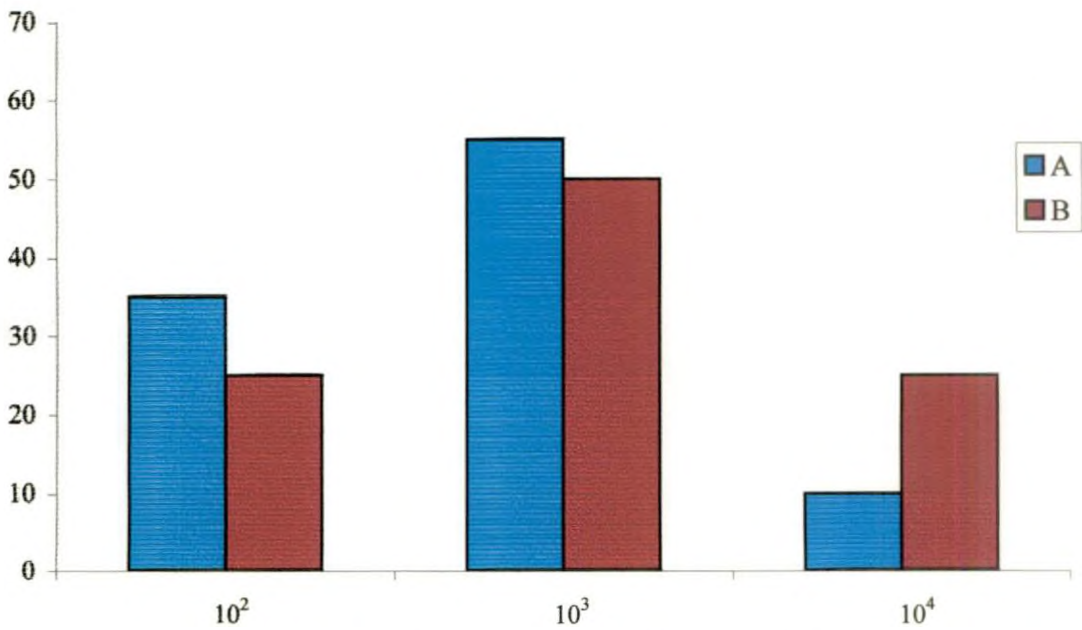

Fig. 2. Distribution of carcasses based on coliform count

source A was at the level of 10^3 cfu /cm^2, while only 50 per cent of samples from source B had the above count. However, the count in 21 (52.5 per cent) out of 40 samples was at the level of 10^3 cfu/cm^2. The counts in 17.5 and 30 per cent of the samples were at the levels of 10^4 and 10^2 cfu/cm^2, respectively.

Table 4. Distribution of carcasses based on coliform count

Source of samples	Coliform count on carcasses (cfu/cm^2)		
	10^2	10^3	10^4
A	7 (35)	11 (55)	2 (10)
B	5 (25)	10 (50)	5 (25)

Figures in parenthesis indicate percent

4.1.3. *Escherichia coli* Count

The mean *Escherichia coli* count of carcasses belonging to sources A and B are given in table 5. Analysis of variance test of the count revealed significant difference (P<0.05) between the mean count of samples from the two sources. The samples belonging to source A had a high mean count. The overall mean *Escherichia coli* count of the samples was 1.83 ± 0.22 $\log_{10}$ cfu/cm^2.

Table 5. The mean *Escherichia coli* count of 40 beef samples belonging to Source A and B

Source of carcass	No. of samples tested	Mean ± SE ($\log_{10}$cfu/cm^2)
A	20	$2.53^{a*} \pm 0.19$
B	20	$1.13^{a} \pm 0.25$
Overall mean	40	1.83 ± 0.22

Figures bearing the same superscript differ significantly
* P<0.05

Distribution of carcasses based on *Escherichia coli* count

The distribution of carcasses based on *Escherichia coli* count is shown in table 6 and illustrated in fig. 3. One of the samples belonging to the source A and nine of the samples from the source B did not reveal the presence of the organism. The count of the samples from both the sources varied from 10^1 to 10^3

cfu/cm^2. The count in 55 per cent of the samples of source A was at the level of 10^2 cfu/cm^2, whereas, only 25 per cent of the samples from the source B had the above count. Of the 40 samples, 16 (40 per cent) had the count at the level of 10^2 cfu/cm^2. The count in 17.5 per cent of the samples was at the level of 10^1 cfu/cm^2, while an equal percent of samples had the count at the level of 10^3 cfu/cm^2.

Table 6. Distribution of carcasses based on *Escherichia coli* count

Source of samples	*Escherichia coli* count on carcasses (cfu/cm^2)		
	10^1	10^2	10^3
A	3 (15)	11 (55)	5 (25)
B	4 (20)	5 (25)	2 (10)

Figures in the parenthesis indicate percent

4.1.4. Faecal Streptococcal Count

The mean faecal streptococcal count of carcasses belonging to the sources A and B are given in table 7. Analysis of variance test of the count revealed significant difference ($P<0.05$) in the mean counts of samples from the two sources. Source A had higher mean count. The overall mean streptococcal count of samples was 3.27 ± 0.10 $\log_{10}$ cfu/cm^2.

Table 7. The mean faecal streptococcal count of samples belonging to source A and B

Source of carcass	No. of samples tested	Mean ±SE ($\log_{10}$ cfu/cm^2)
A	20	$3.55^{a*} \pm 0.11$
B	20	$3.00^{a} \pm 0.10$
Overall mean	40	3.27 ± 0.10

Figures bearing the same superscript differ significantly
* P<0.05

Distribution of carcasses based on faecal streptococcal count

The distribution of carcasses based on the faecal streptococcal count from both the sources is shown in table 8 and illustrated in fig 4. The count on the carcasses from the source A ranged between 10^2 and 10^4 cfu/cm^2, while the count on the carcasses belonging to sources B ranged from 10^2 to 10^3 cfu/cm^2. The count in 30 per cent of the carcasses from the source A was at the level of 10^4 cfu/cm^2, whereas, none of the carcasses belonging to the other source had count at the above level. The count in 50 per cent of the samples from source A was at the level of 10^3 cfu/cm^2. Out of the 40 carcasses 18 (45 percent) had count at the level of 10^3 cfu/cm^2. The counts on 40 and 15 per cent of the carcasses were at the level of 10^2 and 10^3 cfu/cm^2, respectively.

Table 8. Distribution of carcasses based on faecal streptococcal count

Source of samples	Faecal streptococcal count on carcasses (cfu/cm^2)		
	10^2	10^3	10^4
A	4 (20)	10(50)	6(30)
B	12(60)	8(40)	-

Figures in parenthesis indicate percent

4.2. CORRELATION BETWEEN VARIOUS BACTERIAL COUNTS

The association between the mean of different bacterial counts of the samples belonging to the source A is shown in table 9. Analysis of data revealed that the association between the mean count of coliforms and faecal streptococci was significant ($P<0.05$) and had a positive relationship between the count. The relationship between all other bacterial counts were negative and non significant.

Table 9. Correlation coefficient between various bacterial counts of the samples belonging to source A

Bacterial count	Correlation coefficient between bacterial count			
	TVC	CC	ECC	FSC
TVC		-0.10	-0.22	-0.32
CC			-0.21	0.53*
ECC				-0.02

*P<0.05

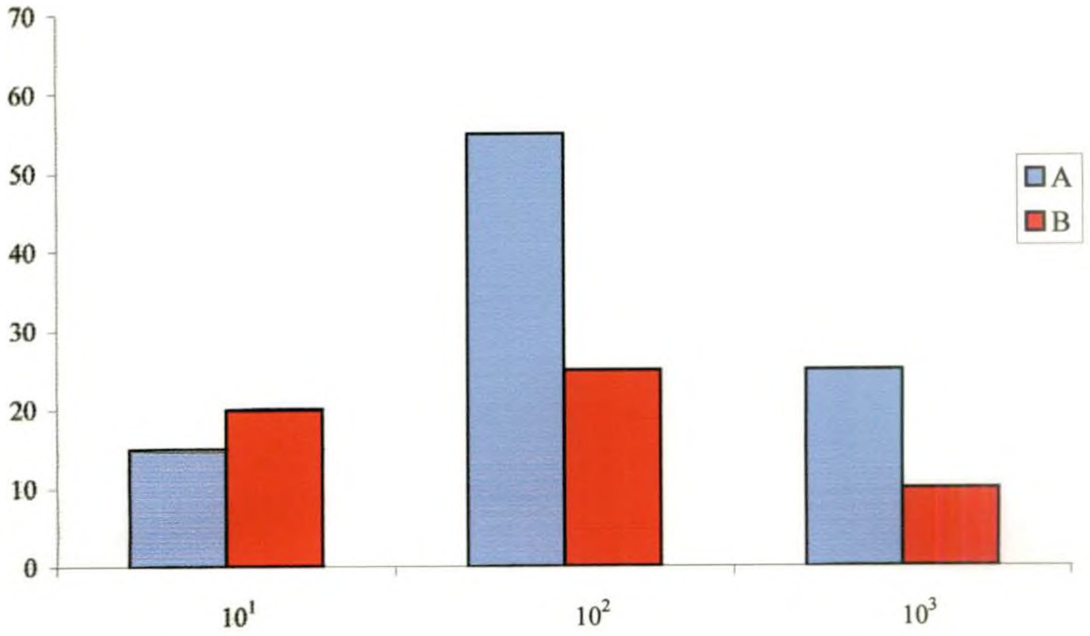

Fig. 3. Distribution of carcasses based on *Escherichia coli* count

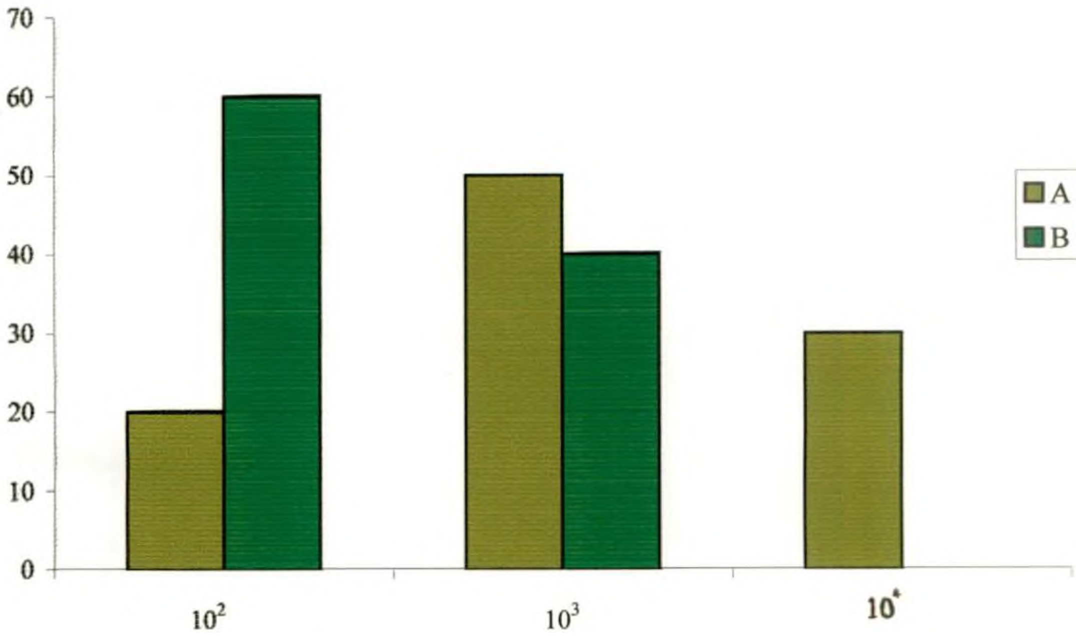

Fig. 4. Distribution of carcasses based on faecal streptococcal count

The correlation coefficient between the mean bacterial count of the samples taken from the source B is given in table 10. A significant and positive association was observed between the mean total viable count and faecal streptococcal count ($P<0.05$). All other bacterial associations were non-significant, but the association between total viable count and coliform count, and coliform count and faecal streptococcal count was positive while the relationship between total viable count and *Escherichia coli* count, coliform count and *Escherichia coli* count and *Escherichia coli* count and faecal streptococcal count were negative.

Table 10. Correlation coefficient between various bacterial counts of the samples belonging to Source B

Bacterial count	Correlation coefficient between bacterial count			
	TVC	CC	ECC	FSC
TVC		0.13	-0.02	0.67*
CC			-0.07	0.37
ECC				-0.02

* $P<0.05$

4.3. ISOLATION AND IDENTIFICATION OF BACTERIA

All samples collected from 40 carcasses, 20 each, obtained from two sources were tested to isolate and identify *Escherichia coli, Staphylococcus aureus*, salmonellae and *Listeria monocytogenes*. The percent of samples positive for the above organism are given in table 12. The results of these studies are as follows.

4.3.1. *Escherichia coli*

Of the 40 samples tested, *Escherichia coli* was isolated from 15 (37.5 per cent) of the samples. Of the isolates, 10 were isolated from the samples belonging to source A and five isolates from the samples collected from the source B. All isolates were identified by the cultural, morphological and biochemical

characteristics described by Barrow and Feltham (1993) and were sent for serotyping at National Salmonella and Escherichia centre, Central Research Institute, Kasauli, Himachal Pradesh. The serotypes isolated from the samples belonging to each source are shown in table 11.

Table 11. Distribution of *Escherichia coli* serotypes isolated from the carcass samples

Serotypes	No. of isolates		Total
	Source A	Source B	
08	1	1	2
013	-	1	1
036	4	-	4
065	-	1	1
069	-	1	1
075	-	1	1
0156	1	-	1
0157	2	-	2
0172	1	-	1
UT	1	-	1

UT = Untypeable

Of the 15, except one, 14 isolates were serotyped and grouped under nine serotypes. Two isolates from source A was serotyped as 0157. Four isolates from this source belonged to serotype 036. The serotypes 036, 0156, 0157, and 0172 were isolated, only from the samples obtained from the source A. The serotype 08 was isolated from the samples collected from both the sources. However, the serotypes 013, 065, 069 and 075 were isolated only from the carcasses belonging to source B.

4.3.2. *Staphylococcus aureus*

All 40-carcass samples were tested for the isolation of *Staphylococcus aureus*. All isolates were identified by the cultural, morphological and biochemical characteristics described by Barrow and Feltham (1993). The organism was isolated only from two (5 per cent) carcasses from source A and one (2.5 per cent) of the carcasses from source B.

4.3.3. Salmonellae

The 40 carcass samples were analysed for the detection of salmonellae. One isolates each from the source A and B was identified as salmonellae based on cultural, morphological and biochemical characteristics described by Edwards and Ewing (1972) and Barrow and Feltham (1993).

4.3.4. *Listeria monocytogenes*

All carcass samples were tested for the isolation and identification of *Listeria monocytogenes,* but none of the samples revealed the presence of this organism.

Table 12. Pathogenic organism isolated from beef carcass samples

Carcass source	No. of samples tested	No. (%) of samples positive for			
		Escherichia coli	*Staphylococcus aureus*	*Salmonellae*	*Listeria monocytogenes*
A	20	10 (50)	2 (10)	1 (5)	ND
B	20	5 (25)	1 (5)	1 (5)	ND
Total	40	15 (37.5)	3 (7.5)	2 (5)	ND

Figures in the parenthesis indicate per cent
ND – not detected

4.4. BACTERIAL LOAD OF PROCESSING EQUIPMENT AND ENVIRONMENTAL SAMPLES

Bacterial load in different processing equipment, ice, water and hand washings of personnel are given in table 13.

Table 13. The mean bacterial load of processing equipment, water, ice and hand washings

Sl. No.	Source	Mean bacterial count ± SE (log_{10} cfu/cm^2 or per ml)			
		TVC	CC	ECC	FSC
1.	Cutting board	4.94 ± 0.87	1.24 ± 0.94	-	1.38 ± 0.86
2.	Cutting table	4.28 ± 0.87	1.26 ± 0.83	-	1.29 ± 0.79
3.	High density polyethylene bags	1.18 ± 0.76	-	-	-
4.	Knife	2.36 ± 0.76	1.11 ± 0.73	-	1.13 ± 0.70
5.	Steel	3.08 ± 0.75	1.57 ± 0.74	-	-
6.	Pond water	5.10 ± 0.74	1.39 ± 0.77	-	-
7.	Tap water	3.24 ± 0.09	0.50 ± 0.21	-	-
8.	Ice	3.20 ± 0.11	2.30 ± 0.08	-	-
9.	Hand wash	4.96 ± 0.82	1.26 ± 0.78	0.80 ± 0.24	1.48 ± 0.77

Highest mean total viable count was observed in pond water. The hand wash of workers had a mean count at the level of 4.96 ± 0.82 log_{10} cfu/ml. The count in meat cutting board and meat cutting table were at the level of 4.94 ± 0.87 and 4.28 ± 0.87 log_{10} cfu/cm^2, respectively. However, the count in the high-density polyethylene bags used as packaging material was the lowest.

Coliforms were observed in all samples except in packaging material. The highest count of the organism was observed in ice samples. *Escherichia coli* count was observed only in hand wash samples. Faecal streptococci count was observed only in cutting board, cutting table, knife and hand wash.

The mean bacterial load of air samples in the slaughter hall and chilling room are shown in table 14. The mean count in the former samples was 100.7 ± 8.17 cfu /min and in the latter samples was 8.75 ± 1.19 cfu /min.

Table 14. The mean bacterial load of air samples in processing plant.

Sampling site	Mean counts ± SE (cfu /min)
Slaughter hall	100.7 ± 8.17
Chilling room	8.75 ± 1.19

Discussion

5. DISCUSSION

In the present study 20 beef carcasses each, brought from sources A and B, were tested to determine the overall bacterial quality of carcasses by estimating total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count. All carcass samples were also examined to determine the presence of *Escherichia coli, Staphylococcus aureus*, salmonellae and *Listeria monocytogenes.* During the investigation the microbial load of environmental samples, processing equipments and hands of personnel involved in various operations were also evaluated.

5. 1. BACTERIAL COUNT

5.1. 1. Total Viable Count

The samples in the present study had an overall mean total viable count of 7.40 ± 0.17 $\log_{10}$ cfu/cm^2. The mean counts obtained from both the sources were almost same and showed no significant difference. The count was in the range of 10^6 to 10^9 $\log_{10}$ cfu/cm^2. According to Ingram (1949), the count of the carcasses produced from abattoir with poor hygienic practices was in the range of 10^6 to 10^7/cm^2. Therefore it may be inferred that the two sources from which the carcasses were produced had a poor hygienic practice. It may also be seen that spoilage of meat is observed in meat stored at 1 or 4°C when the total count of meat reach at the level of 7.2 to 8.0 log/cm^2 (Rao *et al.,* 1998). A great majority of the fresh carcasses tested had count at the above level. The high initial bacterial count of carcasses from both sources may reduce their shelf life. This inference may be conferred with that of the observation of Jay (1996). However, the count obtained in the present investigation was in agreement with that of Bacon *et al.* (2000). But the count was 3 log greater than that reported by Latha (1993), Rajeev (1994) and Ziauddin *et al.* (1994).

Out of 40 carcasses examined, 45 per cent had count at the level of 10^6 cfu/cm^2, which was much greater than the count described by Karr *et al.* (1996) that ranged between 10^3 and 10^4cfu/cm^2. None of the samples belonging to the two sources had count at the level of FSIS microbiological study, which indicated that 93.1 per cent samples had count under 10^4 cfu/cm^2.

The high total viable count (10^9 cfu/cm^2) observed in the present study could be attributed to the lack of hygienic practices followed during slaughter and dressing of cattle and subsequent transportation. An improvement in the hygienic and sanitary practices followed at the various level of slaughter, dressing and processing can produce beef with satisfactory bacterial quality.

5.1.2. Coliform Count

Analysis of variance test of the data revealed that the mean coliform count of the samples belonging to source A were significantly ($P<0.05$) higher than that from source B. The samples had an overall mean coliform count of 3.41 ± 0.31 $\log_{10}$ cfu/cm^2. The count obtained in the study was almost similar to the count recorded by Ziauddin *et al.* (1994), while it is one log higher than that reported by Latha (1993) and Rajeev (1994). The count observed in the samples ranged between 10^2 and 10^4 cfu/cm^2. It is higher when compared to the FSIS (1994) in which 96.4 per cent of the samples had the count under 10^2cfu/cm^2. Majority of samples from source A had count at the level of 10^3 cfu/cm^2. This indicates that the hygienic practices, followed in the above source were not satisfactory. This also confirms the observation of Lasta *et al.* (1992), who reported that carcasses produced from very good abattoirs had the count lower than one cfu/cm^2.

The large number of organism found in the meat samples indicate that the contamination of the carcasses might have occurred from animal's intestinal content, slaughter hall environment, equipment and vehicles used for the transportation of the carcasses.

5.1.3. Escherichia coli Count

The mean *Escherichia coli* count of the samples of the source A was significantly ($P<0.05$) higher than that of the source B. The overall mean count of the organisms in the samples was 1.83 ± 0.22 $\log_{10}$ cfu/cm^2. The count was higher than that reported by Sofos *et al.* (1999) and Martinez *et al.* (2002). However, the organism did not reveal in 25 per cent of the samples. The level of organism in the positive samples varied from 10^1 to 10^3 cfu/cm^2, which is much greater than that of the ≤ 10 cfu/cm^2 reported by the FSIS (1994).

Escherichia coli is a mesophilic, gram-negative organism found in the intestinal tract of man and animals. The organism is associated with spoilage of meat and their presence on the carcasses indicates that the contamination might have occurred from the intestinal content of the animal and from the contaminated water used for various activities during slaughter and dressing of carcasses.

The detection of organism in 47.5 per cent of the carcasses belonging to the source A and in 27.5 per cent of the carcasses belonging to the source B indicates the unsatisfactory hygienic practices followed during the various stages of dressing and transportation.

5.1.4. Faecal Streptococcal Count

The analysis of variance test revealed that the mean count in source A was significantly ($P<0.05$) higher than that of the count of the samples of source B. The overall mean faecal streptococcal count of the samples was 3.27 ± 0.10 $\log_{10}$ cfu/cm^2. The mean count of the samples belonging to the source A was almost similar to the count reported by Ziauddin *et al.* (1994) who observed a count of 3.43 $\log_{10}$/cm^2. The count of the samples in the present study was 1.32 log greater than that reported by Latha and Nanu (1994). All samples revealed the presence of organism and its presence in large number could be attributed to direct or indirect faecal contamination, as these organisms are true commensals of

the alimentary tract of man and animals. At times, the organisms are associated with "sours" or "bone taint" on the carcasses (Jay, 1996). The detection of organism on cent percent samples revealed that the hygienic practices followed during the slaughter and dressing of these animals was not satisfactory.

5.2. ISOLATION AND IDENTIFICATION OF VARIOUS BACTERIA

5.2.1. Escherichia coli

Escherichia coli are mesophilic gram-negative bacteria and are intestinal inhabitants of animals and man. The organism is widely distributed and their presence on carcasses indicates faecal contamination. At times, these commensal organisms are also associated with certain pathogenic conditions in man and animals. In the present study, the organism was isolated from 15 (37.5 per cent) out of 40 samples. The percent of isolation of the organism from the samples belonging to the sources A and B were 50 and 25, respectively. Contamination of the carcasses with presumptive *Escherichia coli* on 17 per cent and 34.4 per cent beef samples was reported by Ingham and Schemidt, (2000).

In the present study, out of the 15 isolates, 14 were serotyped and grouped under nine serotypes. One of the isolate belonging to source A was untypable. The serotype O157 was isolated from two carcasses belonging to the source A, but none of the carcasses from source B had the organism. The isolation of this organism from one of the 255 beef carcasses was reported by Guyon *et al.* (2001). The isolation of the organism from the carcasses is of great significance since the organism is associated with hemolytic colitis, hemolytic uremic syndrome and thrombocytopenic purpura in man. The serotype O156 is an enterohaemorrhagic *E. coli* isolated from one of the carcasses belonging to source A. The serotype O8 which is an enterotoxigenic *E. coli* was isolated from one carcass each belonging to the source A and B, and is the major causative agent of infantile and travellers diarrhoea. The isolation of the serotype from buffalo beef was reported by Banerjee *et al.* (2001). The serotypes O65 and O75 were

isolated from one carcass each of the source B. The former is associated with urinary tract infection and the latter is an enteroaggregative organism. The other serotypes obtained from either of two sources are O13, O36, O69 and O172.

The isolation of *Escherichia coli* from carcasses, which are associated with various diseases in man and animals, is of great significance. Since the organism is of intestinal origin of man and animals, and is considered as an indicator organism the possibility of the presence of various pathogenic organisms cannot be ruled out. Therefore, adequate measures have to be taken to control the contamination of carcasses with such bacterial pathogens.

5.2.2. Staphylococcus aureus

The organism was isolated from 5 per cent of carcasses belonging to source A and 2.5 per cent of carcasses from source B. The findings of the study was much lower than that recorded by Phillips *et al.* (2001) who isolated the organism from 24.3 per cent of sponged beef carcasses and 25.3 per cent of excised carcasses. The isolation of the organism was also reported from 30 per cent of fresh buffalo meat (Bachhil, 1998) and 16.6 per cent of beef samples (Hansson, 2001).

Staphylococcus aureus is generally regarded as a potential pathogen. However, their presence on fresh carcasses can be considered as indicators of poor hygienic practices, since the organism is of ubiquitous nature and are usually the inhabitants of anterior nares, axilla and skin of man and animals.

5.2.3. Salmonellae

Salmonellae were isolated from one of the carcasses each from source A and B. The findings of the study was much higher than that reported by Phillips *et al.* (2001) who reported the isolation of the organism from 0.2 per cent of the sponged samples of beef carcasses and 0.6 per cent of the excised beef carcasses. However, the observation of the present study was much lower than that of the

isolation from 19 per cent (Beach *et al.*, 2002) and 6.7 per cent (Ransom *et al.*, 2002) of beef carcasses.

Isolation of the organism from the carcasses is of great public health significance since the organism is a potential pathogen and is associated with food borne salmonellosis in man.

5.3. PROCESSING EQUIPMENT AND ENVIRONMENTAL SAMPLES

5.3.1. Processing Equipment, Ice, Water and Hand Washing

The mean total viable count on meat cutting board was 4.94 ± 0.87 $\log_{10}$ cfu/cm^2 and the count on meat cutting table was at the level of 4.28 ± 0.87 $\log_{10}$ cfu/cm^2. The mean coliform count on the former was at the level of 1.24 ± 0.94 $\log_{10}$ cfu/cm^2 and the latter had count at the level of 1.26 ± 0.83 $\log_{10}$ cfu/cm^2. The count is almost similar to ≤ 10^2 cfu/cm^2 reported by Karr *et al.* (1996). The mean faecal streptococcal count was at the level of 1.38 and 1.29 $\log_{10}$ cfu/cm^2 on meat cutting board and meat cutting table, respectively. The above mentioned data indicate the significance of meat cutting board and meat cutting table on the contamination of the carcasses and the products prepared from it.

During the transportation of carcasses from the site of production to the plant, ice is used to control the microbial growth on the carcasses. The ice used for the above purpose had a mean total viable count of 3.20 ± 0.16 $\log_{10}$ cfu/ml. The count in the pond and tap water were 5.10 ± 0.74 and 3.24 ± 0.09 $\log_{10}$ cfu/ml, respectively. The mean count in the tap water was much higher than the reported 10 cfu/ml (Rao and Ramesh, 1992) and 2.07 $\log_{10}$ cfu/ml (Tarwate *et al.*, 1993). The level of coliforms in ice, pond and tap water were at the level of 2.30 ± 0.08, 1.39 ± 0.71 and 0.50 ± 0.21 $\log_{10}$ cfu/ml, respectively. The observation of the study indicates that the ice, pond and tap water had contributed significantly in the contamination of beef carcasses.

The personnel engaged in various operations during the processing of carcasses played a significant role in the contamination of carcasses. The mean total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count observed per ml of the hand wash of these employees were 4.96 ± 0.82, 1.26 ± 0.78, 0.80 ± 0.24 and 1.48 ± 0.77 log_{10} cfu, respectively. The detection of *Escherichia coli* and faecal streptococci in the hand wash of the workers indicated the poor hygienic practices of those personnel. Therefore, it may inferred that these employees might also contribute to the contamination of the carcasses along with spoilage and pathogenic organisms particularly of intestinal origin.

5.3.2. Slaughter Hall and Chilling Room

The bacterial level of the air samples in the slaughter hall and chilling rooms were estimated since the beef carcasses brought for processing in the plant were brought in to the slaughter hall, where the carcasses were subjected to washing and trimming and then the selected carcasses were transferred in to the chilling room. Mean bacterial load in the slaughter hall and chilling room obtained in the present study were 100.7 ± 8.17 and 8.75 ± 1.19 cfu/min, respectively. These findings indicate that the air in these room play a significant role in the bacterial contamination of the carcass surface.

Summary

6. SUMMARY

In the present investigation a total of 40 randomly selected beef carcass samples were collected from a meat processing plant located at Kochi in Kerala. The plant procures beef carcasses from two slaughtering units (A and B), located in Tamil Nadu, for fabrication and production of meat and its products. From each source 20 beef carcasses were selected. From each carcass surface 500-cm^2 areas consisted of 100 cm^2 each from neck, brisket, loin, flank and outer round were swabbed with sterile moist cotton swab. The samples collected from the sites of each carcass were pooled and analysed the bacterial quality of the carcasses by estimating total viable count, coliform count, *Escherichia coli* count and faecal streptococcal count. The samples were also tested for the isolation and identification of *Escherichia coli, Staphylococcus aureus,* Salmonella and *Listeria monocytogenes.* The environmental samples such as water and the samples of equipment and hand wash of personnel engaged in the processing plant were also collected and estimated the bacterial load in these samples.

The samples in the present study had an overall mean total viable count of 7.40 ± 0.17 log_{10} cfu/cm^2. The mean counts obtained from both the sources were almost similar, but the samples belonging to the source B had a high mean count. Of the 40 samples examined, one sample of source A had count at the level of 10^9 cfu/cm^2. The count in 45 per cent samples was at the level of 10^6 cfu/cm^2, while the count in 30 per cent and 22.5 per cent were at the levels of 10^8 cfu/cm^2 and 10^7 cfu/cm^2, respectively.

The analysis of variance test of coliform count of carcass samples revealed that the mean coliform count of the samples belonging to source A was significantly ($P<0.05$) higher than that from source B. The samples had an overall mean coliform count of 3.41 ± 0.13 log_{10} cfu/cm^2. The count of the samples from both the sources varied from 10^2 to10^4 cfu/cm^2. The count in 55 per cent of samples from source A was at the level of 10^3 cfu /cm^2, while only 50

per cent of samples from source B had the count at the above level. Of the 40 carcasses tested 52.5 per cent of samples had the count at the level of 10^3 cfu/cm^2. The count in 17.5 and 30 per cent of samples were at the levels of 10^4 and 10^2 cfu/cm^2, respectively.

The mean *Escherichia coli* count of the samples from source A was significantly ($P<0.05$) higher than that of the samples from source B. The samples had an overall mean *Escherichia coli* count of 1.83 ± 0.22 $\log_{10}$ cfu/cm^2. The organism was not detected in 25 per cent of the carcass samples but in the positive samples, the level of organism varied from 10^1 to 10^3 cfu/cm^2. The count in 55 per cent of the samples of source A was at the level of 10^2 /cm^2, where as only 25 per cent of the samples from source B had the count at the above level. The count in 17.5 per cent of the samples was at the level of 10^1 cfu/cm^2, while an equal percent of samples had the count at the level of 10^3 cfu/cm^2.

The analysis of variance test revealed that the mean faecal streptococcal count in source A was significantly ($P<0.05$) higher than that of the count in the samples of source B. The overall mean faecal streptococcal count of the samples was 3.27 ± 0.10 $\log_{10}$ cfu/cm^2. The count on the carcasses from the source A ranged between 10^2 and 10^4cfu/cm^2, while the count on the carcasses belonging to source B ranged from 10^2 to10^3 cfu/cm^2. In 45 per cent of the samples the count was at the level of 10^3 cfu/cm^2.

Analysis of data revealed that the association between the mean count of coliforms and faecal streptococci of the samples belonging to source A was significant ($P<0.05$). A significant ($P<0.05$) association was observed between the means of total viable count and faecal streptococcal count of the samples from source B.

Of the 40 carcass samples tested 15 (37.5 per cent) samples showed the presence of *Escherichia coli*. The organism was isolated from 10 samples

belonging to source A and 5 samples from Source B. These 15 isolates were subjected to serotyping, out of which 14 were grouped under nine serotypes and one was untypable. Two carcass samples from source A showed the presence of serotype O157, which is considered to be the most pathogenic *Escherichia coli* serotype. The serotype O36 was isolated from four samples of this source. Serotypes like O36, O156, O157 and O172 were isolated only from the samples obtained from the source A. Both the sources showed the presence of serotype O8. However, the serotypes O13, O65, O69 and O75 were isolated only from samples of source B.

Staphylococcus aureus was isolated from two (5 per cent) carcasses belonging to the source A and one (2.5 per cent) of the carcasses belonging to the source B.

Of the 40 carcass samples tested salmonellae were isolated from one sample each from the source A and B.

Listeria monocytogenes could not be isolated from any of the carcass samples belonging to source A and B.

Meat cutting board and meat cutting table had a mean total viable count of 4.94 ± 0.87 and 4.28 ± 0.87 $\log_{10}$ cfu/cm^2, respectively. The mean faecal streptococcal count was at the level of 1.38 and 1.29 $\log_{10}$ cfu/cm^2 on meat cutting board and meat cutting table, respectively.

Mean total viable count obtained from ice samples was 3.20 ± 0.11 $\log_{10}$ cfu/ml. The pond water and tap water had the count at the level of 5.10 ± 0.74 and 3.24 ± 0.09 $\log_{10}$ cfu/ml, respectively. The coliform count in ice, pond and tap water were 2.30 ± 0.08, 1.39 ± 0.77 and 0.50 ± 0.21 $\log_{10}$ cfu/ml, respectively.

The hand wash samples of the workers involved in various operations in the plant had a mean total viable count of 4.96 ± 0.82 $\log_{10}$ cfu/ml. The mean

coliforms count, *Escherichia coli* count and faecal streptococcal count per ml of the hand wash samples were 1.26 ± 0.78, 0.80 ± 0.24 and 1.48 ± 0.77 log_{10} cfu/ml, respectively.

The air samples of slaughter hall and chilling room had a mean bacterial load of 100.7 ± 8.17 and 8.75 ± 1.19 cfu/min, respectively.

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EVALUATION OF BACTERIOLOGICAL QUALITY OF BEEF CARCASSES IN MEAT PROCESSING PLANT

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ABSTRACT

During the present study, 40 beef carcasses were randomly selected from a meat processing plant located at Kochi in Kerala. The plant procures beef carcasses from two slaughtering units, viz., Source A and B, located in Tamil Nadu. From each carcass surface 500-cm^2 area was swabbed which consisted of 100 cm^2 each from neck, brisket, loin, flank and outer round. The samples from each carcass were examined for the bacterial quality by estimating the total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC). All samples were also subjected to the isolation and identification of *Escherichia coli*, *Staphylococcus aureus*, Salmonella and *Listeria monocytogenes*. The samples of air, water, equipment and hand wash of personnel were also collected and estimated the various bacterial loads of these samples.

Analysis of variance test of the data did not reveal significant difference between the mean total viable count of the samples from two sources. The samples had an overall mean total viable count of 7.40 ± 0.17 $\log_{10}$ cfu/cm^2. The mean count of samples from source B was slightly higher than that from source A. The count of the samples belonging to source A ranged from 10^6 to 10^9 cfu/cm^2 while the count of the samples from source B varied between 10^6 and 10^8 cfu/cm^2. Of the 40 carcass samples examined, 45 per cent had count at the level of 10^6 cfu/cm^2. The counts in 30 per cent and 22.5 per cent were at the level of 10^8 cfu/cm^2 and 10^7 cfu/cm^2, respectively.

Analysis of variance test of the data revealed a significant ($P<0.05$) difference between the mean coliforms count of the samples from the two sources. The samples from source A had a higher mean count. The overall mean coliforms count of the samples was 3.41 ± 0.13 $\log_{10}$ cfu/cm^2. The count of the samples from both the sources varied from 10^2 to 10^4 cfu/cm^2. The count in 21 (52.5 per cent) carcass samples was at the level of 10^3 cfu/cm^2. In 17.5 per cent

carcasses the count was at the level of 10^4 cfu/cm^2 and in 30 per cent carcass samples the count was at the level of 10^2 cfu/cm^2.

Analysis of variance test of the *Escherichia coli* count revealed a significant (P<0.05) difference between the mean count of samples from the two sources. The samples belonging to source A had a higher mean count. The overall mean *Escherichia coli* count of the samples was 1.83 ± 0.22 $\log_{10}$ cfu/cm^2. The *Escherichia coli* count of the samples from both the sources varied from 10^1 to 10^3 cfu/cm^2. The count in 16 (40 per cent) of carcass samples was at the level of 10^2 cfu/cm^2. In 17.5 per cent samples each had count at the levels of 10^1 and 10^3 cfu/cm^2.

Analysis of variance test of the faecal streptococcal count revealed significant (P<0.05) difference between the mean counts of samples from the two sources. Source A had higher mean count. The overall mean count of samples was 3.27 ± 0.10 $\log_{10}$ cfu/cm^2. The count on the carcasses from the source A ranged between 10^2 to 10^4 cfu/cm^2 whereas the count on the carcasses belonging to source B varied between 10^2 to 10^3 cfu/cm^2. Out of 40 carcasses, 18 (45 per cent) had count at the level of 10^3 cfu/cm^2. The counts on 40 and 15 per cent of the carcasses were at the levels of 10^2 to 10^4 cfu/cm^2, respectively.

A significant (P<0.05) positive correlation was observed between the mean CC and FSC of the carcasses belonging to source A. The association between the mean TVC and FSC in source B was significant (P<0.05).

Escherichia coli was isolated from 10 carcasses belonging to source A and 5 carcasses from source B. Out of the 15 isolates, 14 were serotyped and were grouped under nine serotypes and one isolate was untypeable. The serotype O157 was isolated from two of the carcass samples belonging to source A. Four isolates from this source belonged to serotype O36. The serotypes O36, O156, O157 and O172 were isolated only from the samples obtained from the source A.

The serotype O8 was isolated from both the sources. However, the serotypes O13, O65, O69 and O75 were isolated only from samples of source B.

Staphylococcus aureus was isolated from two (5 per cent) carcasses belonging to source A and one (2.5 per cent) of the carcasses belonging to the source B.

Of the 40 carcass samples tested, salmonellae were isolated from one sample each from the source A and B.

Listeria monocytogenes could not be isolated from any of the samples belonging to both the sources.

The mean total viable counts on meat-cutting board and meat-cutting table were 4.94 ± 0.87 and 4.28 ± 0.87 log_{10} cfu/cm^2, respectively. The mean coliforms count on the former was 1.24 ± 0.94 log_{10} cfu/cm^2 and the latter was at the level of 1.26 ± 0.83 log_{10} cfu/cm^2. The mean faecal streptococcal count was at the level of 1.38 and 1.29 log_{10} cfu/cm^2 on meat cutting board and meat cutting table, respectively.

Ice samples had a mean total viable count of 3.20 ± 0.11 log_{10} cfu/ml. The coliforms count in ice, pond, and tap water were 2.30 ± 0.08, 1.39 ± 0.77 and 0.50 ± 0.21 log_{10} cfu/ml, respectively.

The mean total viable count, coliforms count, *Escherichia coli* count and faecal streptococcal count observed per ml of the hand wash of the personnel engaged in various operations were 4.96 ± 0.82, 1.26 ± 0.78, 0.80 ± 0.24 and 1.48 ± 0.77 log_{10} cfu/ml, respectively.

The mean bacterial load in the air samples of slaughter hall and chilling room obtained in the present study was 100.7 ± 8.17 and 8.75 ± 1.19 cfu/min, respectively.