BACTERIA OF PUBLIC HEALTH SIGNIFICANCE IN BROILER DRESSED CHICKEN

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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KERALA, INDIA

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DECLARATION

I hereby declare that this thesis entitled **"BACTERIA OF PUBLIC HEALTH SIGNIFICANCE IN BROILER DRESSED CHICKEN"** 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.

Mannuthy
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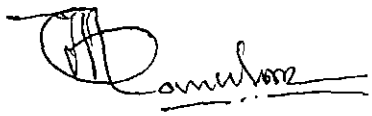
BINDU RAJ .R.

CERTIFICATE

Certified that the thesis entitled **"BACTERIA OF PUBLIC HEALTH SIGNIFICANCE IN BROILER DRESSED CHICKEN"** is a record of research work done independently by **Dr. Bindu Raj .R.**, 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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We, the undersigned members of the Advisory Committee of **Dr. Bindu Raj .R.,** a candidate for the degree of Master of Veterinary Science in Veterinary Public Health, agree that the thesis entitled **"BACTERIA OF PUBLIC HEALTH SIGNIFICANCE IN BROILER DRESSED CHICKEN"** may be submitted by Dr. Bindu Raj .R., in partial fulfilment of the requirement for the degree.

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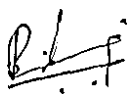

BINDU RAJ. R.

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Introduction

1. INTRODUCTION

In animal agricultural sector, poultry meat trade is one of the successfully growing industries. India has been ranked as the country with the highest livestock population in the world and has been ranked as 19th in broiler production. During 2000, the country had a broiler population of 600 millions, and poultry meat production of 775 thousand tonnes. The growing demand of broiler meat in Kerala is being catered by over 4000 broiler production units located in various parts of the state. These units have a capacity to produce about 200 lakhs of broilers annually which meet about half of the state's needs. The rest of the broiler requirement is being met by the supply of birds from the neighbouring states.

In1999, the poultry meat production of the world was 63.7 million tonnes. The annual per capita chicken meat consumption in India in 1998 was less than one kilogram and that of Kerala about 1.29 kg/year. The rapid urbanization and improvement in the standard of living coupled with change in food habits and life style has led to the incorporation of foods of animal origin in the diet. Meat, particularly chicken forms a part of human diet since it has a high biological value in human nutrition.

In India, less than 2% of the broiler chicken produced is being retailed as dressed or packed and frozen or cut-up-chicken. The majority of consumers prefer to purchase live broiler chicken from the processing units and

get them dressed from the same unit. The consumers prefer this type of retail dressed chicken due to the belief that freshly dressed chicken is qualitatively safe. Generally in chicken stalls and broiler units birds are dressed under unhygienic conditions on planks or wooden blocks. In almost all cases, carcasses are skinned and not defeathered. This type of carcass dressing leads to contamination of the carcass with saprophytic and pathogenic bacteria. The level and extent of bacterial contamination of the carcasses with the former groups of bacteria influence the shelf life of the carcasses. The growth and multiplication of the organisms result in the spoilage of meat. However, contamination of carcasses with the latter type of organism and their growth and multiplication on the carcass do not cause much appreciable changes on the carcass but may lead to food borne infections and intoxications to the consumers. Therefore, frequent bacterial monitoring of the carcasses produced in such retail shops will give an insight on the bacterial profile of the carcasses. An understanding of the bacterial profile of the carcasses is essential for proper application of appropriate sanitary measures and enhancing the hygienic practices to improve the bacterial quality of the carcasses. Therefore the present study was undertaken to assess the bacterial quality of broiler chicken carcasses by

A. Estimating
   1. Total viable count
   2. Coliforms count
   3. *Escherichia coli* count
   4. Faecal streptococcal count

B. Determining the presence of bacterial pathogens such as:

1. *Escherichia coli*
2. Salmonellae
3. *Staphylococcus aureus*
4. *Listeria monocytogenes*

Review of Literature

2. REVIEW OF LITERATURE

2.1 Bacterial count

2.1.1 Total viable count

Panda (1971) evaluated the level and types of bacteria on retail poultry carcasses by swabbing an area of 4 cm^2 on the front, middle and back. The mean total aerobic count of samples collected from shops 1, 2 and 3 located at Bangalore was 2.5 x 10^5, 3.3 x 10^6 and 2.8 x 10^5 per cm^2, respectively. The mean count of the samples obtained from the two shops in Mysore was 3.9 x 10^6 and 5.4 x $10^6/cm^2$, respectively.

Maxcy *et al.* (1973) studied the microbiological quality of minced poultry meat. The total viable count in 7.67, 33.33, 41.03 and 17.93 per cent samples of hand deboned fresh ground poultry meat ranged from 10^3 to 10^4, 10^4 to 10^5, 10^5 to 10^6, 10^6 to 10^7/g, respectively.

Janky *et al.* (1978) compared the microbiological quality of brine chilled, water chilled and hot packaged broiler carcasses. The study revealed that the mean aerobic plate count of the rinse culture before chilling and hot packaging was 6.5 x 10^4/ml.

Mercuri and Cox (1979) examined two samples of broiler chicken skin obtained from local supermarket and reported that the samples had a mean aerobic plate count of 5.8 log/g.

Lillard (1980) studied the effect of treating broiler carcasses with chiller water containing chlorine or chlorine dioxide on the microbial quality of carcasses. The study revealed that the untreated carcasses had a mean aerobic plate count of 4.60 $\log_{10}$/g.

Panda (1980) assessed the bacteriological quality of minced poultry meat and reported that the fresh ground poultry meat had a mean total aerobic count of 3.60 $\log_{10}$ cfu/g.

Reddy and Varadarajulu (1981) studied the relationship between microbial counts, extract release volume and pH of refrigerated chicken. The study revealed that the surface swab samples obtained from the thigh region had a mean total aerobic count of 4.73 log/cm^2.

Cox *et al.* (1983) evaluated the microbiological profile of thigh meat of broilers and reported that the samples had a mean aerobic plate count of 7.0 $\log_{10}$/g.

Anand *et al.* (1987) analysed the bacterial quality of breast, drumstick and under wings of 52 dressed broiler chicken. The total aerobic count of breast ranged from 18 x 10^1 to 34 x 10^3 cfu/cm^2. The count of drumstick and underwing samples ranged between 10 x 10^1 and 17 x 10^2 and 20 x 10^2 and 84 x 10^4 cfu/cm^2 respectively.

Anand *et al.* (1989) analysed the swab samples taken from breast, drumstick, underwings and abdominal cavity of starved and non starved dressed chicken to assess the influence of starving on microbial quality of

chicken carcasses. The mean aerobic plate count of the latter group of chicken carcasses was 3.9, 3.7, 4.7 and 4.9 $\log_{10}$ cfu/cm^2 in the breast, drumstick, underwings and abdominal cavity, respectively. The corresponding count in the former group of chicken carcasses was 2.3, 2.1, 3.9 and 4.4 $\log_{10}$ cfu/cm^2.

Anand *et al.* (1990) studied the microbial profile of culled broiler chicken carcasses and reported the mean total aerobic count of meat as 4.28 $\log_{10}$ cfu/g.

Lillard (1990) studied the impact of commercial processing procedures on the bacterial contamination of broiler chicken carcasses at various stages of dressing by the whole carcass rinse method. The mean aerobic bacterial count per pre chill carcass of the plant A and B was 6.69 and 6.67 $\log_{10}$ cfu, respectively.

Nair *et al.* (1990) evaluated the bacterial quality of dressed broiler chicken obtained from Central Food Technological Research Institute (CFTRI) processing plant and from local market. The total aerobic count was at the level of 10^5/g in all the samples from CFTRI, whereas 48 (96%) samples from the market had count at the level of 10^6/g and the remaining 2 (4%) samples had count at the level of 10^7/g.

Anand *et al.* (1991) studied the quality and shelf life of chicken patties and reported that the manually deboned meat from culled birds had a mean aerobic plate count of 4.3 ± 0.05 log/g.

Fliss *et al.* (1991) assessed the bacterial quality of fresh meat from various species of animals and poultry obtained from different sources. The study revealed that poultry carcasses obtained from the market had a mean total aerobic count of 5.25 $\log_{10}/cm^2$.

Anand *et al.* (1992) investigated the changes in the microflora during preparation and storage of chicken tandoori and reported that the dressed birds had a mean aerobic plate count of 5.36 ± 0.04 log/g.

James *et al.* (1992) estimated the bacterial quality of 160 raw poultry carcasses obtained from a poultry slaughter establishment and reported the samples had a mean aerobic plate count of 3.39 $\log_{10}$ cfu/pre chilled carcass.

Jetton *et al.* (1992) compared the efficacy of physiological saline and distilled water as carcass rinse media for the recovery of total aerobic bacteria, total coliforms, *Escherichia coli* and *Salmonella*, from broiler carcasses. The mean aerobic plate count of physiological saline and distilled water carcass rinse was 3.48 $\log_{10}$ cfu/ml and 3.52 $\log_{10}$ cfu/ml, respectively.

Murugkar *et al.* (1993) examined the microbiological quality of 108 frozen chicken samples comprising 18 samples each of boneless legs, liver, gizzard, kheema, whole dressed chicken and boneless breast procured from poultry processing plant. The study revealed that the whole dressed chicken had a mean aerobic plate count of 5.57 log cfu/g. The total viable count in 11.1 per cent samples was in the range of 3 to 4 log cfu/g. The count in 61.1, 16.3

and 11.1 per cent samples ranged from 4 to 5, 5 to 6, 6 to 7 log cfu/g, respectively.

Whittemore (1993) compared the plate count method, three tube MPN procedure and a modified MPN method to enumerate total aerobes, *Enterobacteriaceae* and salmonellae on broiler poultry carcasses by the whole carcass rinse procedure. The mean aerobic plate count observed in the plate count method, three tube MPN procedure and the modified MPN method was 3.55 ± 0.35, 2.50 ± 0.35 and 4.28 ± 0.52 $\log_{10}$ cfu/ml, respectively.

Abu-Ruwaida *et al.* (1994) assessed the microbiological quality of broilers during processing in a modern commercial slaughter house and reported that the freshly processed carcasses had a mean aerobic count of 6.6 $\log_{10}$ cfu/g of neck skin on the first day samples and on the second day the count on the neck skin was 6.5 $\log_{10}$ cfu/g.

Al-Mohizea *et al.* (1994) evaluated the microbial quality of chilled eviscerated whole chicken broiler carcasses and reported that the samples had a mean initial total aerobic count of 4.67 $\log_{10}/cm^2$.

Vorster *et al.* (1994) analysed the bacterial quality of 43 broiler carcasses by carcass rinse method and reported that the samples had a mean aerobic plate count of 6.8 $\log_{10}$ cfu/ml.

Pattnaik *et al.* (1997) examined the microbial loads on thirty leg meat samples from hot eviscerated (unchilled) carcasses of one month old broiler chicken dressed and sold at local market in Bhubaneswar city. The study

revealed that the chicken leg samples had a mean aerobic plate count of 7.93 $\log_{10}$ cfu/10g.

Bailey *et al.* (2000) studied the microbiological profile of chilled and frozen broiler chicken by carcass rinse method and reported the mean mesophilic aerobic plate count as 4.62 $\log_{10}$ cfu/ml of the carcass rinse.

Berrang *et al.* (2000) analysed the effect of hot water application on the levels of various bacteria on broiler carcasses and reported that the mean total aerobic count of defeathered carcasses was 4.4 $\log_{10}$ cfu/ml of the carcass rinse.

Berrang *et al.* (2001) estimated the presence and level of various bacteria in broiler chicken parts with and without skin. The mean total aerobic count of breast, thigh and drumstick meat of the eviscerated pre-chill broiler cuts was 2.8 ± 0.2, 2.9 ± 0.6 and 3.4 ± 0.3 $\log_{10}$ cfu/part, respectively.

2.1.2 Coliforms count

Panda (1971) evaluated the coli-aerogenes count of retail poultry carcasses and reported that the mean count of the samples from three shops in Bangalore was 73, 93 and 104/cm^2, respectively. The mean count of the samples from two shops in Mysore was 116 and 98/cm^2, respectively.

Maxcy *et al.* (1973) evaluated the microbial quality of 39 samples of minced hand deboned fresh ground poultry meat. The count in 20.51 per cent samples was less than 10/g. The count in 28.21, 25.64, 23.08 and 2.56

per cent of samples varied from 10 to 99, 100 to 999, 10^3 to 10^4 and more than 10^4/g, respectively.

Janky *et al.* (1978) studied the sensory, physical and microbiological quality of brine chilled, water chilled and hot packaged broiler carcasses. The study revealed that the mean faecal coliforms count of carcasses before chilling and hot packaging was 2.2 x 10^4/ml of the carcass rinse.

Mercuri and Cox (1979) examined two samples of broiler chicken skin obtained from local supermarket and reported that one of the samples had a count of log 2.0/g and the count of the other sample was log 1.8/g.

Lillard (1980) analysed the effect of chlorine and chlorine dioxide in chiller water on the microbiological quality of broiler carcasses. During the study, it was observed that the untreated carcasses had a mean faecal coliforms count of 3.07 $\log_{10}$/g of breast skin.

Panda (1980) studied the bacteriological quality of minced poultry meat and reported that fresh ground poultry meat had a mean coliforms count of 1.23 $\log_{10}$/g.

Cox *et al.* (1983) estimated the microbiological quality of chicken patty products and observed that the thigh meat used for the preparation of the patties had a coliforms count greater than 3.4 $\log_{10}$/g.

Anand *et al.* (1987) investigated the bacterial quality of 52 fresh dressed broiler chicken. The study revealed that the non-faecal coliforms count

of the samples taken from the breast ranged between zero and 4 x 10^1 cfu/cm^2. The count of the samples from drumstick ranged from zero to 2 x 10^2 cfu/cm^2 and the count of the underwing samples ranged between zero and 12 x 10^2 cfu/cm^2. The coliforms count ranged between 4 x 10^1 and 12 x 10^2/cm^2 in 22 per cent of the samples.

Anand *et al.* (1989) assessed the microbial quality of breast, drumstick, underwings and abdominal cavity of chicken carcasses derived from non starved and starved chicken. The mean coliforms count of the breast, drumstick, underwings and abdominal cavity of the former group of chicken carcasses was 1.9, 3.1, 3.3 and 3.5 $\log_{10}$ cfu/cm^2, respectively. The corresponding count of the samples form latter group was 1.1, 2.0, 2.7 and 2.9 $\log_{10}$ cfu/cm^2.

Anand *et al.* (1990) studied the microbial profile of chicken sausages and reported that the culled broiler chicken carcasses used for the preparation of the sausage had a mean coliforms count of 1.84 log/g.

Nair *et al.* (1990) studied the microbial quality of dressed broiler birds procured from the local market and the poultry processing unit of Central Food Technological Research Institute (CFTRI). The coliforms count was observed at the level of 10^2/g in 20 (40%) samples. The count in 15 (30%) of samples, each, obtained from CFTRI, was at the level of 10^3 and 10^4/g, respectively. The samples belonging to the local market had count at the level of 10^3, 10^4 and 10^5/g is 14, 80 and six per cent of the samples.

Anand *et al.* (1991) analysed the microbial quality and shelf life of chicken patties and reported that the manually deboned meat from culled birds had a mean coliforms count of 2.41 ± 0.04 $\log_{10}$ cfu/g.

Fliss *et al.* (1991) studied the microbiological quality of fresh meat from various species of animals and poultry. The study indicated that the poultry carcasses obtained from the markets had a mean coliforms count of 3.3 $\log_{10}/cm^2$.

Anand *et al.* (1992) evaluated the changes in the microflora of chicken tandoori during its preparation and storage. They reported that the dressed birds had a mean coliforms count of 3.05 ± 0.05 $\log_{10}$ cfu/g.

Jetton *et al.* (1992) compared the efficacy of physiological saline and distilled water as broiler carcass rinse media for the recovery of bacterial populations. The mean total coliforms count in saline rinse was 2.26 $\log_{10}$ cfu/ml, and the corresponding count in the distilled water rinse was 2.11 $\log_{10}$ cfu/ml.

Abu-Ruwaida *et al.* (1994) assessed the microbial quality of broiler chicken carcasses during processing of chicken in a modern commercial slaughter house. They observed that the neck skin of freshly processed carcasses of first day of sampling had a mean coliforms count of 4.1 log cfu/g and the count of the samples collected on second day was 4.9 log cfu/g.

Al-Mohizea *et al.* (1994) examined the quality of chilled eviscerated whole chicken broiler carcasses and reported that the samples had a mean coliforms count of 2.21 $\log_{10}/cm^2$.

Pattnaik *et al.* (1997) assessed the microbial quality of 30 samples of leg meat from hot eviscerated (unchilled) carcasses of one month old broiler chicken, dressed and sold at local market of Bhubaneswar city and reported that the samples had a mean coliforms count of 7.22 $\log_{10}/10g$.

Bailey *et al.* (2000) studied on the bacterial quality of unchilled, chilled and frozen broiler chicken carcasses by the rinse method and reported that the unchilled carcasses had a mean coliforms count of 2.25 $\log_{10}/ml$.

Berrang *et al.* (2000) evaluated the effect of hot water application on the levels of various bacteria on broiler carcasses. The study revealed that the carcasses after defeathering had a mean coliforms count of 3.3 $\log_{10}$ *cfu*/ml of the whole carcass rinse.

Berrang *et al.* (2001) estimated the presence and level of different types of bacteria on broiler parts with and without skin. The result of the study indicated that the breast, thigh and drumstick meat without skin had a mean coliforms count of 2.2 ± 0.4, 1.8 ± 0.7 and 2.3 ± 0.4 $\log_{10}$ *cfu*/part, respectively.

2.1.3 *Escherichia coli* count

Panda (1980) assessed the bacteriological quality of minced poultry meat and reported that the samples had a mean *Escherichia coli* count of 1.12 $\log_{10}$ cfu/g.

Cox *et al.* (1983) studied the microbiological profile of the raw ingredients used for the manufacture of chicken patties and reported that the thigh meat used for the preparation of the product had a mean *Escherichia coli* count of 0.8 $\log_{10}$/g.

Nair *et al.* (1990) compared the microbial quality of dressed broilers carcasses obtained from Central Food Technological Research Institute (CFTRI) processing plant and from local market. The *Escherichia coli* count of the samples collected from CFTRI processing plant was at the level of 10^1, 10^2 and 10^3/g in two, 64 and 10 per cent of the samples respectively. However, the organism could not be isolated from 12 of the 50 samples. The samples belonging to the local market had counts at the level of 10^2, 10^3 and 10^4/g in 16, 68 and 16 per cent respectively. The count of the organism in eight out of 50 local market samples was at the level of 10^2/g. The count of 34 samples was at the level of 10^3/g and in eight samples the count was at the level of 10^4/g.

Fliss *et al.* (1991) evaluated the bacterial quality of fresh meat from various species of animals and poultry from different sources. The mean *Escherichia coli* count of the poultry carcasses from the market was 0.76 $\log_{10}/cm^2$.

James *et al.* (1992) examined the bacterial profile of 160 raw poultry carcass rinse samples and observed that the samples had a mean *Escherichia coli* count of 1.46 $\log_{10}$ cfu/carcass.

Jetton *et al.* (1992) evaluated the efficiency of physiological saline and distilled water as carcass rinse media and reported that the recovery rate of *Escherichia coli* from broiler carcasses with physiological saline was 1.53 $\log_{10}$ cfu/ml and the recovery rate with distilled water was 1.63 $\log_{10}$ cfu/ml.

Abu-Ruwaida *et al.* (1994) evaluated the microbiological quality of broilers during processing in a modern commercial slaughter house and reported that the neck skin of the freshly processed carcasses had a mean *Escherichia coli* count of 3.6 $\log_{10}$ cfu/g.

Vorster *et al.* (1994) examined the bacterial quality of 43 broiler carcasses and observed that the samples had a mean *Escherichia coli* MPN count of 2.4 $\log_{10}$ cfu/ml.

Bailey *et al.* (2000) assessed the bacterial profile of chilled and frozen chicken employing the whole carcass rinse method. The samples had a mean *Escherichia coli* count of 1.96 $\log_{10}$ cfu/ml.

Berrang *et al.* (2000) studied the effect of hot water application on broiler carcasses and its effect on various bacteria. During the investigation

it was observed that the defeathered carcasses had a mean *Escherichia coli* count of 2.7 $\log_{10}$ cfu/ml.

Berrang *et al.* (2001) investigated the presence and level of various bacteria from broiler parts with and without skin. The study revealed that the breast, thigh and drumstick meat without skin had a mean *Escherichia coli* count of 1.6 ± 0.2, 1.8 ± 1.0 and 2.1 ± 0.4 log cfu/part, respectively.

2.1.4 Faecal streptococcal counts

Panda (1971) assessed the microbial quality of retail poultry carcasses by swabbing an area of 4 cm^2 on the front, middle and back. The mean faecal streptococcal count of the carcasses from the three shops at Bangalore was at the level of 1.2 x 10^3, 3.2 x 10^4 and 1.9 x $10^3/cm^2$, respectively. The corresponding count of the samples from the two shops in Mysore was 1.7 x 10^4 and 2.7 x $10^3/cm^2$.

Cox *et al.* (1983) studied the bacteriological quality of raw ingredients of chicken patty products and reported that the thigh meat used for patty preparation had a streptococcal count of 3.5 $\log_{10}$/g.

Anand *et al.* (1990) studied the microbial profile of chicken sausages during its preparation and storage. During the investigation it was observed that the dressed broilers used for the preparation of sausage had a mean faecal streptococcal count of 2.30 log/g.

Anand *et al.* (1991) evaluated the microbial quality and shelf life of chicken patties. The study revealed that the manually deboned meat from culled birds had a mean faecal streptococcal count of 2.09 ± 0.04 log/g.

Anand *et al.* (1992) analysed the changes in bacterial quality during preparation and storage of chicken tandoori and observed that the dressed birds had a mean faecal streptococcal count of 2.97 ± 0.07 log/g.

2.2 Isolation of bacteria

2.2.1 *Escherichia coli*

Panda (1971) tested the bacterial quality of dressed chicken obtained from three market shops in Bangalore and two shops in Mysore and reported the isolation of *Escherichia coli* from four per cent of the samples.

Black *et al.* (1981) isolated *Escherichia coli* serotypes 078:H10, 078:H11, 078:H12 and 078:H18 from the stool culture of patients with diarrhoea. Among the patients, three (10%) had entero toxigenic *Escherichia coli* (ETEC) infection who had contact with water source or animals which were ETEC positive.

The enterotoxigenic *Escherichia coli* (ETEC) are of public health significance since they are associated with diarrhoea in infants, young children and adults in developing countries (WHO scientific working group, 1981). They are also responsible for 60 to 70 per cent of the diarrhoeal episodes in travellers from industrialized countries to the developing world.

Sherwood *et al.* (1985) reported the isolation of Verocytotoxic serotypes of *Escherichia coli* from diarrhoeic calves and the serotypes included 04, 08, 019, 026, 0111, 0149 and 0168.

Bok *et al.* (1986) studied the incidence of various pathogens on 102 retail broiler carcass rinse and reported that 6.85 per cent isolates were *Escherichia coli.*

Doyle and Schoeni (1987) assessed the presence of Vero cell cytotoxic *Escherichia coli* 0157:H7 in retail fresh meat and poultry meat samples. The organism was isolated from four (1.5%) of the 263 poultry meat samples.

Blanco *et al.* (1988) isolated 289 *Escherichia coli* from 78 diarrhoeic calves. One of the isolates was a stable toxin (Sta) producing serotype 09:K(A)35. Verotoxin producing serotypes such as 02, 0103, 0104, 0128, 0153, 0157:H7 and 24 untypables were isolated from 16 calves. The cytotoxic necrotizing factors (CNF) producing serotypes 015, 078, 0123 and 0139 were also isolated from 14 calves.

Panneerselvam *et al.* (1988) isolated 72 *Escherichia coli* strains from 440 swabs collected from ailing and recently dead birds. The serotypes 02, 06, 08, 017, 029, 037, 038, 043, 051, 060, 061, 070, 081, 085, 091, 0120, 0121, 0123, 0124, 0135, 0137, 0148, 0154 and 0156 were isolated from cases of colisepticemia. The serotypes isolated from egg peritonitis included 06, 038, 061, 070, 078, 091, 0120, 0123, 0130 and 0143. The *Escherichia coli* serotypes

isolated from birds suffering from enteritis included 02, 06, 037, 038, 060 and 065. The serotype 02 was isolated from chronic respiratory disease and 02 and 038 were isolated from cases of omphalitis.

Hefnawy and Sabah (1990) evaluated the bacterial quality of 30 samples of ready to eat poultry. During the investigation, *Escherichia coli* were isolated from 10 per cent of the samples.

Char and Rao (1991) reported the isolation of *Escherichia coli* serotypes 02, 08, 018, 020, 024, 026,044, 045, 055, 086, 0111, 0119 and 0125 from poultry suffering from various disease conditions.

Gross (1991) reported that *Escherichia coli* serotypes 01, 02 and 078 were found to be responsible for colisepticemia in chicken characterized by air sacculitis, pericarditis and perihepatitis.

Turtura (1991) estimated the microbiological quality of poultry samples obtained from a slaughter house. During the investigation, 259 coliforms were isolated of which 65 were *Escherichia coli*.

Reddy *et al.* (1994) examined heart blood swab, intestinal swab, yolk material and cut sections of liver obtained from chicken with colisepticemia, enteritis, omphalitis and necrotic hepatitis. During the study, 22 *Escherichia coli* were isolated belonging to the serotypes 09, 019, 020, 054, 057, 060, 065, 068, 078, 080, 089, 091, 0101, 0109, 0116, 0148 and 0162.

Samadpour *et al.* (1994) studied the occurrence of shiga-like toxin producing *Escherichia coli* in retail fresh sea food and various meats. The organism was isolated from four (12%) out of 33 chicken samples.

Vorster *et al.* (1994) examined 43 broiler rinse samples and isolated *Escherichia coli* from 34 (79.1%) samples.

Sharma *et al.* (1995a) analysed meat from various species of animals and chicken for the presence of *Escherichia coli* and the organism was isolated from 14 (60.87%) of the 23 samples. The serotypes isolated from the samples include 05, 062, 0158, 0162 and rough and untypable.

Garabal *et al.* (1996) serotyped 1334 *Escherichia coli* isolated from pigs with diarrhoea or oedema disease. The prevalent serotypes associated with diarrhoea in pigs were 08, 09, 020, 0101, 0138, 0141 and 0149 and the serotypes isolated from pigs with oedema disease were 08, 0101, 0138, 0149 and 0157. The serotypes 08, 09, 020 and 0101 were isolated from healthy pigs.

Geornaras *et al.* (1996) studied the bacterial population associated with poultry carcasses and reported that 31 per cent of the 142 isolates were *Escherichia coli.*

The *Escherichia coli* serotypes are classified into enteroaggregative *Escherichia coli* (EAggEC), enteropathogenic *Escherichia coli* (EPEC), enteroinvasive *Escherichia coli* (EIEC), entero haemorrhagic *Escherichia coli* (EHEC) and enterotoxigenic *Escherichia coli* (ETEC) (Jay,

1996). The enterohaemorrhagic *Escherichia coli* (EHEC) are responsible for the production of haemolytic uremic syndrome characterized by haemolytic anaemia, thrombocytopenia and acute renal failure in human beings and also haemorrhagic colitis. The enteroaggregative *Escherichia coli*, enteropathogenic *Escherichia coli* and enteroinvasive *Escherichia coli* have also been associated with diarrhoea in human beings.

Pattnaik *et al.* (1997) examined 30 samples of leg meat from hot (unchilled) eviscerated broiler carcasses and reported four per cent of the 50 isolates to be *Escherichia coli*.

Altwegg and Bockemuhl (1998) have reported that serotypes 0157:H$^-$ and 0157:H7 caused enterohaemorrhagic diarrhoea in all age groups of young children leading to haemolytic uremic syndrome.

Hang'ombe *et al.* (1999) tested 382 samples of broiler chicken carcasses and reported the isolation of *Escherichia coli* from 56.5 per cent of the carcasses.

Heuvelink *et al.* (1999) examined rectal contents and tonsils from Dutch slaughter pigs collected immediately after slaughter and faecal material from poultry layer flocks and turkey flocks for the presence of Verocytotoxin (VT) producing *Escherichia coli* (VTEC) of serogroup 0157. The organism was isolated from two (1.4%) of the 145 pigs. None of the faecal samples from chicken flocks had revealed the presence of the organism, whereas six (1.3%) out of 459 pooled faecal samples from turkey flocks yielded the organism. One

isolate each from porcine and turkey were found potentially pathogenic for humans, based on cytotoxicity test on vero cells.

Sharada *et al.* (1999) isolated 65 strains of *Escherichia coli* from 85 tissue samples collected from birds with different pathological conditions. These isolates belonged to serotypes 03, 04, 05, 011, 025, 035, 037, 051, 061, 079, 084, 098, 0102, 0106, 0111, 0117, 0126, 0138, 0152 and 0165. These serotypes were isolated from perihepatitis cases. Serotypes 01, 08, 010, 011, 022, 035, 048, 051, 079, 095, 0102, 0111, 0115, 0140 and 0152 were isolated from birds with enteritis. The serotypes 02,05, 078, 079, 0109, 0111, 0117 and 0120 were associated with pericarditis and the serotype 011 was isolated from pneumonitis cases.

Banerjee *et al.* (2001) estimated the presence of Verotoxin producing *Escherichia coli* (VTEC) in 109 food samples. During the investigation, *Escherichia coli* was isolated from 12 (44.44%) of the samples. Of the isolates, one was serotype 086 which is Verotoxin producing *Escherichia coli.*

Dutta *et al.* (2001) examined 50 faecal samples from diarrhoeic unweaned piglets and reported the isolation of *Escherichia coli.* The isolates consisted of the predominant serotypes 06, 08,011, 035, 048, 051, 063, 064, 088, 089,0101, 0149, 0157 and 0162.

2.2.2 *Salmonella*

Panda (1971) reported the isolation of *Salmonella* from six per cent of the retail poultry swab samples collected from the carcasses belonging to three shops in Bangalore and two shops in Mysore city.

Jayaraman *et al.* (1972) examined heart blood, liver, intestinal contents, droppings or rectal swab and meat samples from poultry and reported the isolation of *S. newport* from one of the four meat samples.

Al-Hindawi and Rished (1979) investigated various food samples including 47 raw chicken meat samples for the presence and distribution of *Salmonella* spp. in these foods. The study revealed the isolation of salmonellae from six (12.8%) raw chicken meat samples.

Mercuri and Cox (1979) examined two samples each of broiler skin, chicken pot pie, mechanically deboned poultry meat, ground beef and pork sausage and found that the former sample was free from salmonellae.

Al-Rajab and Hussain (1982) analysed 50 samples each of chicken skin and chicken wings for the presence of salmonellae. The organisms were isolated from 18 per cent of the former samples and 20 per cent of the latter samples. The serotypes isolated from chicken skin included *S. saintpaul, S. java, S. typhimurium, S. montevideo, S. anatum and S. muenchen*. The serotypes isolated from chicken wings consisted of *S. infantis, S. emek, S. kottbus* and *S. enteritidis*.

Yagoub and Mohamed (1987) assessed 80 samples of poultry carcass drip and 66 samples of the skin of dressed poultry carcasses for the presence of salmonellae. The organism was isolated from three samples each of drip water and skin samples. The serotypes isolated from carcass drip consisted of *S. emek* and S.16:L,V:monophas and that from skin samples was *S. emek* and *S. heidelberg*.

Boer and Hahne (1990) examined 81 samples of chicken products consisting of drumstick, liver and fillet and reported the isolation of salmonellae from 44 (54%) samples.

Dominguez *et al.* (1990) isolated salmonellae from 125 (62.5%) of the 200 poultry carcasses. The most frequent serotype isolated was *S. typhimurium*.

Nair *et al.* (1990) examined dressed broiler birds obtained from local market and poultry processing unit of Central Food Technological Research Institute. Salmonellae were isolated from two (4%) of the 50 samples from the former source and all samples belonging to the latter source were found to be free from the organism. The serotypes isolated were *S. virchow* and *S. newport*.

Izat *et al.* (1991) studied the incidence, number and serotypes of *Salmonella* on two brands of commercially grown and processed broiler chicks and a third brand of organic broiler chicken. The organism was isolated from five out of 24 carcass rinse from one of the commercially grown group of birds

and six out of 24 birds in the other group. The organism was also isolated from 10 out of the 24 samples collected from organic broilers. The important serotypes isolated from the samples include *S. typhimurium, S. paratyphi* and *S. arizonae.*

Turtura (1991) examined slaughtered poultry carcasses for the detection of *Enterobacteriaceae* and other gram negative bacteria and reported that none of the samples revealed the presence of salmonellae.

James *et al.* (1992) evaluated the prevalence of salmonellae on 160 raw poultry carcass rinse obtained from a poultry slaughter establishment and reported the isolation of the organism from 48 per cent of the prechill carcasses.

Paturkar *et al.* (1992) investigated the prevalence of salmonellae in 96 meat samples comprising beef, mutton, pork and chicken and reported the isolation of *S. butantan* from one chicken sample.

Jerngklinchan *et al.* (1994) assessed the occurrence of salmonellae in 705 raw broiler chicken meat and observed that 467 (66%) of the samples had the organism. The common serotypes isolated from the samples consisted of *S. blockley, S. virchow, S. enteritidis, S. hadar and S. paratyphi* B.

Tadesse and Cizek (1994) analysed a total of 235 samples consisting of swabs, rinse and organs of freshly dressed broiler carcasses to detect the presence of salmonellae. During the investigation the organism was found in 35.4, 19.7 and 31.0 per cent of carcass rinse, carcass swab and organ

tissues, respectively. The most frequent serotypes isolated from the sample were *S. saintpaul* and *S. enteritidis*.

Plummer *et al.* (1995) studied the level of *Salmonella* contamination in 325 retail chicken and 35 giblet samples. The level of contamination in supermarket chicken, fresh chilled and frozen, and chicken from local butcher shops was 18.6, 25.5 and 24.5 per cent of the samples, respectively.

Sharma *et al.* (1995b) evaluated the occurrence of *Salmonella* serovars in foods of animal origin and reported the isolation of the organism in eight out of the 76 frozen dressed chicken samples. The serovars isolated were *S. typhimurium, S. saintpaul, S. indiana* and *S. stanley*.

Rusui *et al.* (1996) estimated the prevalence of *Salmonella* in broiler chicken carcasses retailed at wet markets and processing plants. Salmonellae were isolated from 158 (35.5%) out of 445 carcasses from wet markets and 52 (50%) out of 104 carcasses obtained from processing plants. The major serotypes isolated from the former source consisted of *S. muenchen, S. kentucky, S. enteritidis, S. blockley and S. chincol* and from the latter source include *S. blockley, S. bradford, S. kentucky and S. agona*.

Cason *et al.* (1997) studied the relationship between aerobic bacteria, *Salmonella* and *Campylobacter* on broiler carcasses. Salmonellae were isolated from 7 (23%) of the 30 post pick carcass rinse.

Pattnaik *et al.* (1997) examined 30 samples of leg meat from hot eviscerated carcass of one month old broilers to estimate the bacterial load and also to isolate various bacterial organisms. During the study none of the samples had revealed the presence of salmonellae.

Zivkovic *et al.* (1997) examined chicken meat on carcasses, dressed chicken meat and chicken liver for the detection of *Salmonella* serovars. Salmonellae were detected in 3.87 per cent of chicken meat on carcass, 11.54 per cent of dressed chicken meat and 23.11 per cent of chicken liver. The most common serotypes isolated from the samples include *S. typhimurium, S. hadar* and *S. virchow.*

Telo *et al.* (1998) analysed 80 samples of poultry meat and related products imported in Albania. *Salmonella* were isolated from seven chicken meat, two turkey meat and also from one of the turkey liver samples. The important sertoype isolated was *S. enteritidis.*

Uyttendaele *et al.* (1998) assessed the prevalence of salmonellae in poultry carcasses and their products during four consecutive years from 1993-1996. They reported isolation of salmonellae from 19.4 per cent of the carcasses in 1993, 24.1 per cent in 1994, 21.9 per cent in1995 and 36.7 per cent in 1996. The predominant serotypes isolated during the study were *S. enteritidis, S. hadar* and *S. virchow.*

Duffy *et al.* (1999) studied the incidence of *Salmonella* spp. on Irish retail meat products. The organisms were isolated from 28 (26.4%) 106

poultry meat samples. The common serotypes isolated from chicken samples in the order of preference include *S. bredeney, S. kentucky, S. enteritidis, S. london and S. schwartzangram.*

Hang'ombe *et al.* (1999) investigated the chicken carcasses leaving a poultry processing plant to retail outlets to determine bacterial profile of the carcasses. Salmonellae were isolated from 20.53 per cent of the carcasses and belonged to the serotypes *S. mbandaka* (82), *S. enteritidis* (18), *S. infantis* (2), *S. gallinarum* (2) and rough types (3).

Uyttendaele *et al.* (1999) evaluated 772 samples of poultry carcasses and their products sold in retail market. Of the 133 chicken carcasses examined, salmonellae were present in 45 samples.

Chang (2000) studied the prevalence of *Salmonella* spp. in poultry broilers and shell eggs in Korea. The study revealed the presence of salmonellae in 25.9 per cent of the samples and the serotypes consisted of *S. enteritidis, S. virchow* and *S. virginia.*

Harrison *et al.* (2001) investigated the incidence of salmonellae and *Campylobacter* in raw whole chicken from supermarket and butcher's shop and reported the isolation of salmonellae from 33 per cent and 24 per cent of the samples from the former and latter sources, respectively.

2.2.3 *Staphylococcus aureus*

Panda (1971) examined the microbial quality of retail dressed chicken carcasses obtained from shops supplying birds immediately after

dressing and reported the isolation of *Staphylococcus* from 12 per cent of the samples.

Nkanga and Uraih (1981) studied the prevalence of *Staphylococcus aureus* in various meat samples obtained from traditional markets and reported the isolation of the organism from 54.1 per cent of the 24 frozen chicken samples.

Bok *et al.* (1986) analysed the incidence of food borne pathogens on 102 retail broiler samples. During the investigation they isolated 31 staphylococci of which 19 per cent were coagulase positive.

Vorster *et al.* (1994) estimated the bacteriological quality of 43 broiler carcass rinse samples and reported the isolation of *Staphylococcus aureus* from 17 (39.5%) samples.

Pattnaik *et al.* (1997) investigated the microbial quality of 30 leg meat samples obtained from hot (unchilled) eviscerated carcasses of one month old broiler chicken and reported that 14 (28%) of the isolates were *Staphylococcus aureus.*

Hang'ombe *et al.* (1999) studied the bacterial profile of 382 broiler chicken carcasses. During the study 13 staphylococci were isolated, of which eight were coagulase positive

2.2.4 *Listeria monocytogenes*

McClain and Lee (1988) reported the isolation of *Listeria monocytogenes* from seven of the 22 frozen poultry samples.

Pini and Gilbert (1988a) examined 50 samples each of fresh and frozen raw chicken and reported the isolation of *Listeria monocytogenes* from 33 (66%) of the fresh chicken samples and 27 (54%) of the frozen samples. Other *Listeria* spp. such as *Listeria welshimeri, Listeria seeligeri* and *Listeria innocua* were also isolated from 28 per cent of the samples.

Pini and Gilbert (1988b) compared two procedure for the isolation of *Listeria monocytogenes* from raw chicken and soft cheese samples. The organism was isolated from 70 of 160 chicken samples.

Bailey *et al.* (1989) studied 90 broiler chicken carcasses obtained from three processing plants and reported the isolation of *Listeria monocytogenes* from 21 (23%) of the carcasses.

Genigeorgis *et al.* (1990) examined 60 samples each of fresh turkey wings, drumstick and tails and reported the isolation of *Listeria* spp. from 27 (45%), 17 (28.3%) and 14 (23.3%) of wings, drumstick and tail, respectively. *Listeria monocytogenes* was detected in 20 per cent of the wings, 13.3 per cent of the drumstick and 11.7 per cent of tail. *Listeria innocua* was detected in 5 per cent of wing samples but none of the drumstick and tail samples revealed the presence of the organism. *Listeria welshimeri* was detected in 20 per cent of wings, 15 per cent of drumstick and 11.7 per cent of the tails.

Seneviratna *et al.* (1990) examined bovine, ovine, avian, procine, kangaroo and horse meat to determine the presence of *Listeria* spp. and

reported the isolation of *Listeria monocytogenes* from one of the avian meat samples and *Listeria innocua* from three samples.

Varabioff (1990) evaluated 48 fresh chicken carcasses during various stages of processing to detect the presence of Listeria and reported the isolation of *Listeria innocua* from two of the 12 eviscerated samples. The samples were free of *Listeria monocytogenes.*

Wong *et al.* (1990) estimated the incidence of *Listeria monocytogenes* in various food including 16 chicken carcasses and reported the isolation of the organism from 50 per cent of the chicken carcasses.

Rahmat *et al.* (1991) assessed the prevalence of *Listeria monocytogenes* in 16 poultry samples obtained from four different wet markets. The organism was isolated from six samples.

Kwiatek *et al.* (1992) examined a total of 593 samples of raw meat and poultry for the presence of *Listeria* spp.. During the investigation *Listeria monocytogenes* was isolated from 36 (60%) out of 60 poultry meat samples.

Wang *et al.* (1992) tested pork, beef, lamb and chicken samples to isolate *Listeria* organisms. During the study, *Listeria* spp. were isolated from 11 (52.4%) of the 21 chicken samples and one of the isolates was *Listeria monocytogenes.*

Ojeniyi *et al.* (1996) examined 320 whole ready to cook chicken to detect the presence of *Listeria monocytogenes* and reported the isolation of the organism from 29 (9.1%) of the samples.

Uyttendaele *et al.* (1999) investigated the incidence of various bacteria on different poultry products. During the study they isolated *Listeria monocytogenes* from 15 (11.3%) of the 133 chicken samples.

Choi *et al.* (2001) examined 30 chicken samples to determine the incidence of *Listeria* spp. and found the organism in 10 samples. The isolate consisted of *Listeria monocytogenes, Listeria seeligeri, Listeria welshimeri* and *Listeria innocua.*

Miettinen *et al.* (2001) investigated the prevalence of *Listeria monocytogenes* in legs, drumstick, breast and wings of broiler chicken carcasses. Out of the 61 samples examined 38 (62%) had revealed the presence of the organism.

Soriano *et al.* (2001) evaluated the presence of *Listeria* spp. in five samples each of raw and ready to eat chicken. *Listeria grayi* was isolated from one of the raw chicken samples.

Materials and Methods

3. MATERIALS AND METHODS

In the present investigation an attempt was made to evaluate the bacterial quality of broiler chicken grown, dressed and retailed locally and also grown outside the state and brought to local market, dressed and retailed. These carcasses were also subjected to the isolation of various bacterial pathogens. During the study 60 broiler carcasses were collected, which consisted of 20 each from retail shop which process the broiler chicken brought from outside the state (A), local farmer who rear broiler chicken and sell as dressed carcass (B) and the dressed chicken carcass from Kerala Agricultural University Poultry Farm (C).

3.1 Collection of samples

At a time two dressed whole carcasses were collected from a source. Each carcass was transferred into a clean dry polyethylene bag and the open end of the bag was closed with a rubber band and was transported immediately to the laboratory in a thermocool container. In the laboratory each carcass was processed for the bacteriological examination.

3.2 Processing of samples

The whole carcass rinse method was employed for the evaluation of bacterial quality of the carcass and also for the isolation of the bacteria. The carcass rinse was prepared according to the method prescribed by Cox *et al.* (1981). The polyethylene bag containing each chicken carcass was opened and

the tip of the neck of each carcass was caught with a sterile forceps and sterile normal saline solution was poured on the neck of the carcass and simultaneously scraped with a sterile non-absorbent cotton swab so as to extricate the bacteria attached on the carcass surface into the diluent. In this way the entire carcass was washed and for washing a carcass 100 ml of the diluent was used. After thorough rinsing, the carcass was held at a lifted position in the polyethylene bag so as to enable to drift the carcass rinse into the bag. Then the carcass rinse was transferred into a sterile conical flask and was used for the evaluation of the bacterial count per ml and for the isolation of various bacterial pathogens.

3.3 Dilution of carcass rinse

In order to estimate various bacterial counts, carcass rinse prepared from each carcass was diluted as described by James *et al.* (1992). From each carcass rinse, 10 ml was transferred into 90 ml of sterile physiological saline so as to form one in 10 dilution. From this further 10 fold serial dilutions were made by transferring one ml of the first dilution into 9 ml of normal saline. From each sample, dilutions were made upto 10^9. From these dilutions selected dilutions were used for estimating bacterial count, depending upon the type of bacteria to be counted.

3.4 Bacterial count

3.4.1 Total viable count

Total viable count (TVC) of each sample was estimated by pour plate technique described by Swanson *et al.* (2001). From the selected 10 fold

dilutions of each sample, one ml of the inoculum was transferred onto duplicate petri-dishes of uniform size. To each of the inoculated plates, about 15-20 ml sterile molten standard plate count agar (SPCA) (Hi-media) maintained at 45°C was poured. The inoculum was mixed with the medium by gentle rotatory movement of the inoculated petri-dishes in clockwise, anticlockwise, forward and backward manner. The inoculated plates were allowed to solidify at room temperature and were then incubated at 37°C for 24 h aerobically. At the end of incubation period, petri-dishes with a bacterial count between 30 and 300 colonies were selected and count of each petri-dish was taken with the help of a colony counter. The number of colony forming units per ml of the carcass rinse was calculated by multiplying the mean colony count of duplicate plates with dilution factor and the count per ml of the carcass rinse was expressed as $\log_{10}$ cfu/ml.

3.4.2 Coliforms count

Coliforms count (CC) was estimated according to the procedure described by Nordic Committee on food analysis (1973). From the selected dilution, 0.1 ml of the inoculum was inoculated onto duplicate plates of violet red bile agar (VRBA) (Himedia) and the inoculum was uniformly distributed on the medium with a sterile "L" shaped glass rod and the plates were incubated at 37°C for 24 h. At the end of incubation, purplish red colonies with a diameter of atleast 0.5 mm, surrounded by a reddish zone of precipitate were counted as coliforms. The number of organisms per ml of the sample was estimated by

applying the dilution factor on the mean count of duplicate plates and the count per ml of carcass rinse expressed as $\log_{10}$ cfu/ml.

3.4.3 *Escherichia coli* count

The *Escherichia coli* count (ECC) per ml of the carcass rinse was estimated as prescribed by Indian standards (1980). The count was estimated by inoculating 0.1 ml of the inoculum from selected dilution onto duplicate plates of Eosin Methylene Blue (EMB) agar (Hi-media) and the inoculum was uniformly distributed, over the medium as described in coliforms count. The inoculated plates were incubated at 37°C for 24 h. After the incubation period, colonies with a dark center and a distinct indelible ink, greenish black metallic sheen on deflected light was counted as *Escherichia coli.* The number of organisms per ml of sample was estimated as described for CC and the count per ml of the carcass rinse was expressed as $\log_{10}$ cfu/ml.

3.4.4 Faecal Streptococcal Count

Faecal Streptococcal Count (FSC) of the sample was estimated by the standard procedure described by Nordic Committee on food analysis (1968b). To estimate the number of organisms in the sample, 0.1 ml of the inoculum from the selected dilution was transferred onto duplicate plates of Karl Friedrich (KF) streptococcal agar (Hi-media) and the inoculum was uniformly distributed onto the media as described in CC. The inoculated plates were incubated at 37°C for 48 h. After the incubation period, pink to dark red colonies with a diameter between 0.5 and 3 mm and surrounded by a narrow

whitish zone were counted as faecal streptococci. The number of organisms per ml of the carcass rinse was estimated and expressed as described in CC.

3.5 Isolation and identification of bacteria

Each carcass rinse was subjected for the isolation and identification of *Escherichia coli*, salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes.*

3.5.1 *Escherichia coli*

In order to isolate *Escherichia coli*, a loopful of the carcass rinse was inoculated on to duplicate plates of Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. After the incubation period, three or four colonies with the characteristic of *Escherichia coli* was transferred onto nutrient agar slants and incubated at 37°C overnight. At the end of the incubation period, the inoculated tubes were stored at refrigeration temperature for further characterization and identification of the isolates by cultural, morphological and biochemical reactions described by Barrow and Feltham (1993) and are shown in flow chart 1. All isolates were also subjected to Eijkman test. The isolates identified as *E. coli* were serotyped at National Salmonella and Escherichia center, Central Research Institute, Kasauli.

Flowchart 1. Isolation and identification of *Escherichia coli* from chicken carcass

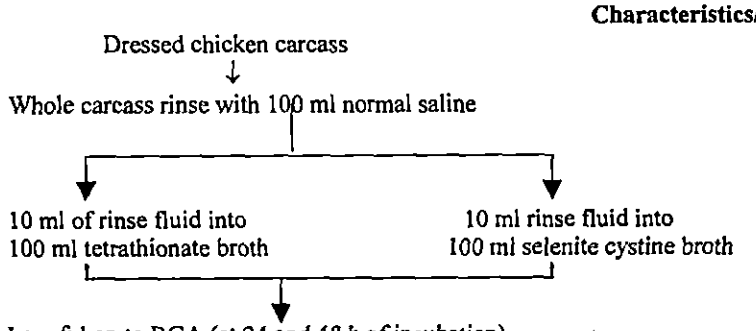
	Characteristics/Reactions
Dressed chicken carcass	
↓	
Whole carcass rinse with 100 ml normal saline solution	
↓	
Inoculate a loopful of the rinse on EMB agar	
↓	
Select suspected colonies	*Colonies with dark center with* distinct indelible ink, greenish black metallic sheen on deflected light
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram negative rods
↓	
Motility	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
OF	F
↓	
Urease	-
↓	
ONPG	+
↓	
Indole	+
↓	
MR	+
↓	
VP	-
↓	
Citrate	-
↓	
Carbohydrate utilization	
Lactose	+
Gluose	+
Mannitol	+
Maltose	+
Inositol	-

F = fermentation; + = positive reaction ; - = negative reaction

3.5.2 *Salmonella*

In order to isolate salmonellae from each carcass rinse, 10 ml each of the carcass rinse was transferred into 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 both 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 24 and 48 h of incubation, a loopful of the culture from each of tetrathionate broth and selenite cystine broth was inoculated onto duplicate plates of Brilliant Green Agar (BGA) (Hi-media) and incubated at 37°C for 24 h. At the end of incubation, colourless pink-white opaque to translucent colonies with a diameter of about 1-2 mm, surrounded by a pink or red hue were selected. 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 flowchart 2.

Flow chart 2. Isolation and Identification of *Salmonella* from chicken carcass



	Characteristics/Reactions
Loopful on to BGA (at 24 and 48 h of incubation)	
↓	
Select suspected colonies	
↓	
Nutrient agar slants	
↓	
Gram's reaction and cell morphology	Gram negative small rods
↓	
Motility	+/-
↓	
Catalase	+
↓	
Oxidase	-
↓	
OF	F
↓	
Urease	-
↓	
TSI	Alkaline slant, acid butt, with/without H_2S and gas
↓	
Decarboxylase → Lysine	+
Decarboxylase → Ornithine	+
↓	
Phenyl alanine deaminase	-
↓	
Indole	-
↓	
MR	+
↓	
VP	-
↓	
Citrate	+/-
↓	
ONPG	-
↓	
Carbohydrate utilization	
Glucose	+
Maltose	+
Mannitol	+
Lactose	-
Sucrose	-
Salicin	-

F = fermentation ; + = positive reaction; - = negative reaction

3.5.3 *Staphylococcus aureus*

For the isolation of *Staphylococcus aureus*, a loopful of the carcass rinse was inoculated onto Staphylococcus Medium 110 (SM: 110) and were incubated at 37°C for 48 h (Nordic Committee on Food Analysis, 1968a). At the end of incubation, colonies showing characteristic appearance (round, smooth, glistening, opaque, convex, amorphous, entire edge and of yellow to golden yellow colour) on SM:110 were selected and transferred to nutrient agar slants and incubated at 37°C for overnight. The isolates were stored at refrigeration temperature for further characterization and identification of the isolates following the procedure described by Barrow and Feltham (1993) and are shown is the flow chart 3. The isolates were identified based on the cultural, morphological and biochemical characteristics.

Flow chart 3. Isolation and identification of *Staphylococcus aureus* from chicken carcass

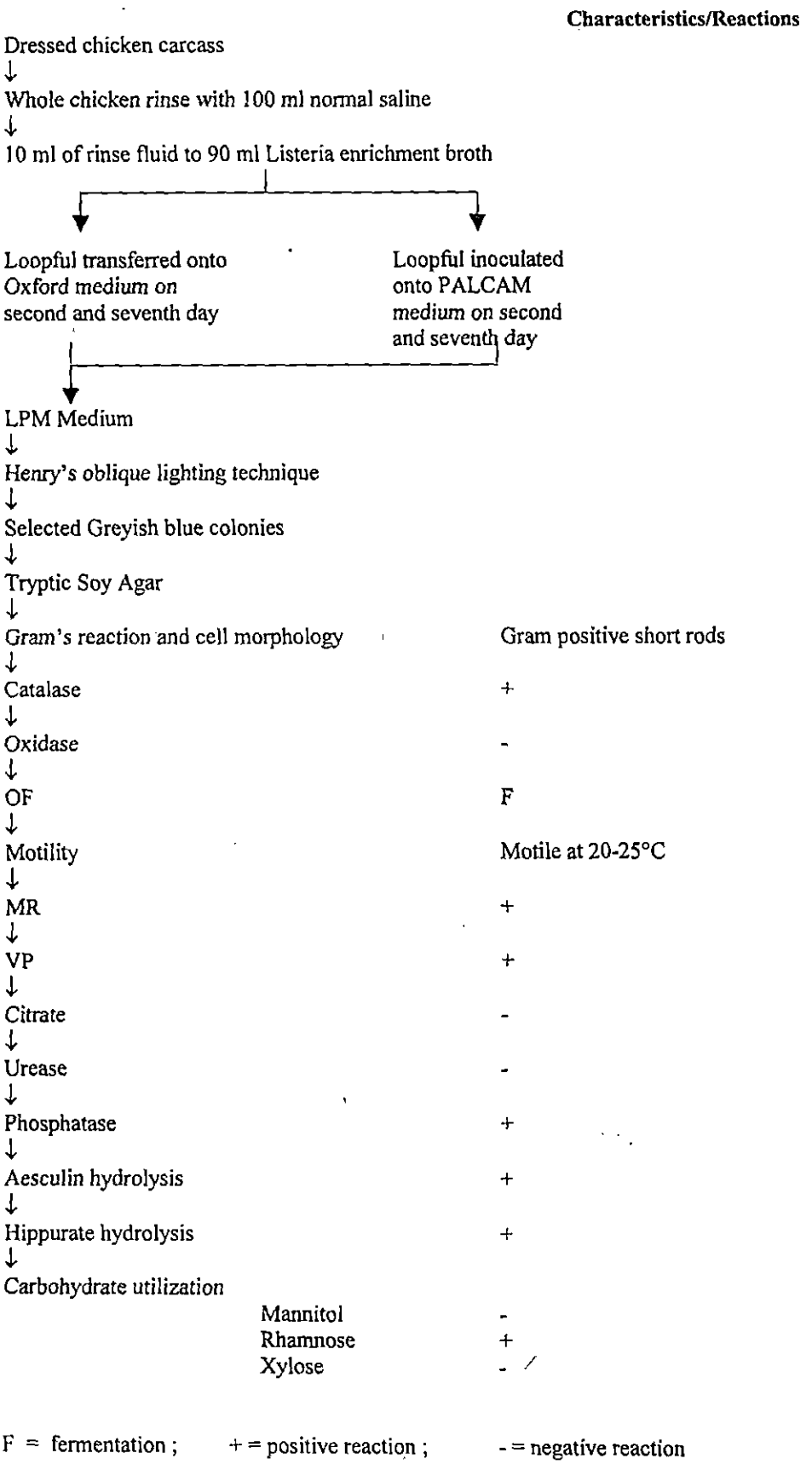
	Characteristics/Reactions
Dressed chicken carcass	
↓	
Whole carcass rinse with 100 ml normal saline	
↓	
A loopful to SM:110 medium	
↓	
Select suspected colonies	Round, smooth, glistening, opaque, convex, amorphous, entire edge, yellow to golden yellow
↓	
Nutrient agar	
↓	
Gram's reaction and cell morphology	Gram positive cocci
↓	
Motility	-
↓	
Growth aerobic	+
↓	
Growth anaerobic	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
OF	F
↓	
VP	+
↓	
Arginine hydrolysis	+
↓	
Phosphatase	+
↓	
Gelatin liquefaction	+
↓	
Urease	+
↓	
Coagulase	+
↓	
Carbohydrate utilization	
Glucose	+
Lactose	+
Sucrose	+
Maltose	+
Mannitol	+
⊢ Aerobic	+
⊢ Anaerobic	+

F = fermentation; + = positive reaction; - = Negative reaction

3.5.4 *Listeria monocytogenes*

From the whole carcass rinse, 10 ml of the fluid was transferred into a sterile conical flask containing 90 ml of Listeria enrichment broth (Bailey et al., 1990), mixed thoroughly and incubated at 30°C for 7 days. A loopful of the inoculum was streaked onto duplicate plates of Oxford agar and Polymyxin B Acriflavin Lithium chloride Ceftazidime Aesculin Mannitol (PALCAM) agar on the second and seventh day of incubation and incubated at 30°C for 24-48 h. At the end of incubation, those colonies with a black zones on Oxford agar and those with cherry red background on PALCAM agar were inoculated onto Lithium chloride Phenylethanol Moxalactam (LPM) agar. The plates were incubated at 30°C for 24 h and examined under Henry's oblique lighting technique. Typical greyish blue colonies were picked and inoculated onto Trypticase Soy Agar (TSA) slants and incubated at 30°C for 24 h and stored at 4°C for further characterization of the isolates. The isolates were subjected to various tests prescribed by Wang *et al.* (1992) as shown in the flowchart 4. The isolates showing characteristics similar to that in flowchart were considered *Listeria monocytogenes*.

Flowchart 4. Isolation and identification of *L. monocytogenes* from chicken carcass



F = fermentation ; + = positive reaction ; - = negative reaction

3.6 Characterization and identification of isolates

The isolates were identified according to the procedures described by Barrow and Feltham (1993) except for the triple sugar iron test.

3.6.1 Primary identification tests

1. Catalase test

Slide test

A small quantity of the colony was transferred on to a clean, grease free, glass slide and mixed well with a drop of three per cent hydrogen peroxide. Evolution of effervescence within a few seconds indicate a positive reaction.

Tube test

One ml of three per cent 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.

2. Gram staining

The procedure for gram staining is as follow:

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 counterstained with dilute carbol fucshin for 30 seconds.

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

3. Motility test

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

4. Oxidase test

A filter paper strip is moistened with a few drops of an aqueous solution of 1% 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 second indicate a positive reaction.

5. Oxidation – Fermentation test

Each isolate was inoculated into 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 upto 14 days. A change in colour of the medium from green to yellow in the open tube 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 indicate no action on carbohydrates.

3.6.2 Secondary tests

1. Aesculin hydrolysis

The organism was inoculated into 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.

2. Arginine hydrolysis

The organism was inoculated into 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 a brown colour.

3. Carbohydrate utilization test

Each isolate was inoculated into two test tubes containing peptone water with Andrade's indicator and one percent 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 centimetre above the media.

4. Citrate utilization 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 upto 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.

5. Coagulase test

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 is rabbit plasma. A positive result is indicated by macroscopic clumping within five seconds and delayed clumping is considered as a negative reaction.

Tube test

Mixed 0.5 ml 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.

6. Decarboxylase reactions

Each isolate was heavily inoculated with a straight wire into three test tubes containing decarboxylase media. One of the tubes contain lysine and other contain ornithine. The third tube is 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.

7. Eijkman test

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

8. Gelatin hydrolysis/liquefaction

Each isolate was inoculated into nutrient gelatin and incubated at 37°C upto 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.

9. 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.

10. Indole production

The isolate was inoculated into peptone water and incubated at 37°C for 48 h. 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.

11. 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.

12. ONPG (O-nitrophenyl-β-D-galactopyranoside) test.

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

13. Phenyl alanine deamination

The phenyl alanine 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% 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.

14. Phosphatase test

The phenolphthalein 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.

15. Triple Sugar Iron Test

Each isolate was stab inoculated into 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).

16. Urease Activity

Slopes 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 indicates a positive reaction.

17. 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% α naphthol

solution and 0.2 ml of 40% aqueous potassium hydroxide into the tube. After thorough mixing of the contents, the tube was kept in a slanting position and examined after 15 minutes and one hour. A positive reaction is indicated by the development of a strong red colour.

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

Results

4. RESULTS

In the present investigation, 60 chicken carcasses, consisting of 20 each, obtained from stalls which bring broiler chicken reared outside the state and selling them as dressed chicken (A), stalls rearing broiler chicken locally and selling them as dressed chicken (B) and dressed chicken carcasses from the Kerala Agricultural University Poultry Farm (C) were evaluated for their bacterial quality by estimating Total Viable Count (TVC), Coliforms Count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC). The carcasses were also tested for the isolation and identification of food borne pathogens such as *Escherichia coli*, salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes*.

4.1 Bacterial count

4.1.1 Total viable count

The mean total viable count (TVC) of samples from the three sources is given in Table 1.

Table 1. Mean total viable count of chicken carcasses from the three sources

Source of carcass	Bacterial count (Mean ± SE $\log_{10}$ cfu/ml)
A	7.94 ± 0.08
B	8.06 ± 0.11[a]
C	7.69 ± 0.13[a]
Overall	7.89 ± 0.07

N = 20, from each source
Figures bearing the same superscript differ significantly

Analysis of variance test of the data revealed significant difference ($P<0.05$) between the mean count of the samples from the source B and C. The overall mean count of samples from the three source was 7.89 ± 0.07 $\log_{10}$ cfu/ml of carcass rinse. Samples belonging to the source B had the highest mean count (8.06 ± 0.11 $\log_{10}$ cfu/ml).

4.1.1.1 Distribution of carcasses based on total viable count

The distribution of broiler chicken carcasses obtained from the three sources based on total viable count/ml of the carcass rinse are shown in Table 2.

Table 2. Distribution of chicken carcasses from the three sources based on total viable count

Source of carcasses	Bacterial count/ml of carcass rinse (cfu/ml)			
	10^6	10^7	10^8	10^9
A	-	$11_{(55)}$	$9_{(45)}$	-
B	-	$11_{(55)}$	$8_{(40)}$	$1_{(5)}$
C	$3_{(15)}$	$12_{(60)}$	$5_{(25)}$	-

N = 20, from each source
Figures in parenthesis indicate per cent

The count of the 60 chicken carcasses ranged between 10^6 and 10^9 cfu/ml of carcass rinse. One (1.67%) of the samples had count at the level of 10^9 cfu/ml of carcass rinse whereas three (5%) carcass rinse had count as low as 10^6 cfu/ml. However, 34 (56.67%) carcass rinse had count at the level of 10^7 cfu/ml and 22 (36.67%) had the count at the level of 10^8 cfu/ml. The rinse samples of all carcasses belonging to the source A and B had count greater than 10^6 cfu/ml. However none of the carcass rinse obtained from the source A and C had the count

at the level of 10^9 cfu/ml, and only 5 (25%) of 20 carcass rinse from source C had count at the level of 10^8 cfu/ml.

4.1.2 *Coliforms count*

The mean coliforms count (CC) of chicken carcasses from the three sources is given in the Table 3.

Table 3. Mean coliforms count of chicken carcasses from the three sources

Source of carcass	Coliforms Count (Mean ± SE $\log_{10}$ cfu/ml)
A	5.30 ± 0.10^{a}
B	5.23 ± 0.13^{b}
C	4.37 ± 0.17^{ab}
Overall	4.97 ± 0.10

N = 20, from each source
Figures bearing the same superscript differ significantly.

Analysis of variance test of the data revealed significant ($P<0.05$) difference between the mean count of the carcasses belonging to the source A and C and B and C. The overall mean count of the carcass rinse was 4.97 ± 0.10 $\log_{10}$ cfu/ml. The carcass rinse belonging to the source A had the highest mean count (5.30 ± 0.10 $\log_{10}$ cfu/ml) and lowest count was observed in the carcass rinse of the samples from the source C (4.36 ± 0.17 $\log_{10}$ cfu/ml).

4.1.2.1 *Distribution of carcasses based on coliforms count*

The distribution of chicken carcasses from the three sources based on coliforms counts are shown in Table 4.

Table 4. Distribution of chicken carcasses from the three sources based on coliforms count

Source of carcass	Coliforms count/ml of carcass rinse (cfu/ml)				
	10^2	10^3	10^4	10^5	10^6
A	-	-	$5_{(25)}$	$14_{(70)}$	$1_{(5)}$
B	-	-	$7_{(35)}$	$11_{(55)}$	$2_{(10)}$
C	$1_{(5)}$	$7_{(35)}$	$7_{(35)}$	$5_{(25)}$	-

N = 20, from each source
Figures in parenthesis indicate per cent

The count of the carcass rinse ranged from 10^2 to 10^6 cfu/ml. All samples from source A and B had count greater than 10^3 cfu/ml. None of the carcass rinse of the samples belonging to the source C had count at the level of 10^6 cfu/ml. Of the samples, 30 (50%) had count at the level of 10^5 cfu/ml of carcass rinse whereas only 3 (5%) samples had the count at the level of 10^6 cfu/ml. The count in 31.67, 11.67 and 1.67 per cent of the carcass rinse were 10^4, 10^3 and 10^2 cfu/ml, respectively. In 14 (70%) of the carcass rinse of the samples from the source A, the count was at the level of 10^5 cfu/ml and 35% samples each from the source B and C had count at the level of 10^4 cfu/ml.

4.1.3 *Escherichia coli* count

The mean *Escherichia coli* count (ECC) of samples from the three sources is given in Table 5.

Table 5. Mean *Escherichia coli* count of chicken carcasses from the three sources

Source of carcass	Bacterial Count (Mean ± SE $\log_{10}$ cfu/ml)
A	2.83 ± 0.48^{a}
B	2.66 ± 0.46^{b}
C	1.08 ± 0.35^{ab}
Overall Mean	2.20 ± 0.27

N = 20, from each source
Figures bearing the same superscript differ significantly.

Analysis of variance test of the data revealed significant ($P<0.05$) difference between the mean *Escherichia coli* count of the samples from source A and C and B and C. The overall mean ECC observed in the carcass rinse of 60 chicken carcasses was 2.20 ± 0.27 $\log_{10}$ cfu/ml. Only 51.67% of the samples revealed the presence of the organism. The organism could not be detected in 9 (45%), 7 (35%) and 13 (65%) chicken carcass from the source A, B and C, respectively. The highest mean count was observed in samples from source A (2.83 ± 0.48 $\log_{10}$ cfu/ml) and lowest in the samples belonging to the source C (1.08± 0.35 $\log_{10}$ cfu/ml).

4.1.3.1 Distribution of carcasses based on *Escherichia coli* count

The distribution of broiler chicken carcasses, obtained from the three sources, based on ECC per millilitre of carcass rinse is shown in Table 6.

Table 6. Distribution of chicken carcasses from the three sources based on *Escherichia coli* count

Source of carcass	ECC/ml of carcass rinse (cfu/ml)		
	10^2	10^3	10^4
A	-	$3_{(15)}$	$8_{(40)}$
B	$1_{(5)}$	$5_{(25)}$	$7_{(35)}$
C	$2_{(10)}$	$4_{(20)}$	$1_{(5)}$

N = 20, from each source
Figures in parenthesis indicate per cent

The data revealed that the count of the carcass rinse varied between 10^2 and 10^4 cfu/ml. Of the samples, 26.67, 20 and five per cent carcass rinse samples had count at the level of 10^4 , 10^3 and 10^2 cfu/ml, respectively. None of

the carcass rinse samples from source A had count at the level of 10^2 cfu/ml, but 40% samples belonging to this source had count at the level of 10^4 cfu/ml whereas only five per cent of the samples from source C had the above count.

4.1.4 Faecal Streptococcal Count

The mean faecal streptococcal count (FSC) of chicken carcasses from the three sources is given in Table 7.

Table 7. Mean faecal streptococcal count of chicken carcasses from the three sources

Source of carcass	Bacterial Count (Mean ± SE $\log_{10}$ cfu/ml)
A	4.99 ± 0.10^{a}
B	4.20 ± 0.10^{ab}
C	3.75 ± 0.10^{ab}
Overall	4.32 ± 0.09

N = 20, from each source
Figures bearing the same superscript differ significantly

Analysis of variance test of the count revealed highly significant ($P<0.01$) difference between the mean count of the carcass from source A and B, A and C and B and C. All samples belonging to the three sources had revealed the presence of the organism. Samples from the source A had the highest mean count and the lowest was in samples belonging to the source C.

4.1.4.1 Distribution of carcasses based on faecal streptococcal count

The distribution of chicken carcasses according to the count of the organism are depicted in Table 8.

Table 8. Distribution of chicken carcasses from the three sources based on faecal streptococcal count

Source of carcass	FSC/ml of carcass rinse (cfu/ml)		
	10^3	10^4	10^5
A	-	$11_{(55)}$	$9_{(45)}$
B	$8_{(40)}$	$12_{(60)}$	-
C	$15_{(75)}$	$5_{(25)}$	-

N = 20, from each source
Figures in parenthesis indicate per cent

The data revealed that the counts of the carcass rinse vary between 10^3 and 10^5 cfu/ml. Only the chicken carcass belonging to the source A had the count at the level of 10^5 cfu/ml of carcass rinse and 55 percent of the samples of this source had count at the level of 10^4 cfu/ml. None of the carcasses from the source B and C had count at the level of 10^5 cfu/ml of the carcass rinse. In 75% of the carcasses from the source C the count was at the level of 10^3 cfu/ml of carcass rinse. The count in 28 (46.67%) of the 60 carcass rinse sample was at the level of 10^4 cfu/ml. The count in 38.33 and 15 per cent of samples were at the level of 10^3 cfu/ml and 10^5 cfu/ml, respectively.

4.2 Correlation between the mean bacterial count

The correlation coefficient between the mean bacterial count of 60 chicken carcasses are shown in Table 9.

Table 9. Correlation coefficient between the mean bacterial count of chicken carcasses

Bacterial count	Correlation coefficient between mean count			
	TVC	CC	ECC	FSC
TVC		0.208	0.058	0.267
CC			0.284	0.642*
ECC				0.210

* $P<0.05$

The correlation coefficient test of the data revealed that all bacterial count of the samples had positive association, but only a significant ($P<0.05$) association was observed between CC and FSC.

4.2.1 Correlation between the mean bacterial count of the samples from source A

The correlation coefficient between the mean bacterial count of the carcasses from source A are shown in Table 10.

Table 10. Correlation coefficient between the mean bacterial count of the carcasses from source A.

Bacterial count	Correlation coefficient between mean count			
	TVC	CC	ECC	FSC
TVC		0.320	0.018	0.682*
CC			0.093	0.140
ECC				-0.217

* $P<0.05$

Analysis of the data revealed a significant ($P<0.05$) and positive correlation between the mean TVC and FSC. However, a positive but non significant association exist between TVC and CC, TVC and ECC, CC and ECC and CC and FSC. A negative and non significant correlation was observed between ECC and FSC.

4.2.2 Correlation between the mean bacterial count of the samples from source B

The correlation coefficient between the bacterial count of the carcasses belonging to the source B are shown in Table 11.

Table 11. Correlation coefficient between the mean bacterial count of the carcasses from source B

Bacterial count	Correlation coefficient between mean count			
	TVC	CC	ECC	FSC
TVC		0.047	-0.001	0.317
CC			0.01	0.618*
ECC				0.115

* P <0.05

A significant (P<0.05) and positive correlation exist between the mean CC and FSC. A positive and non significant correlation was found between TVC and CC, TVC and FSC, CC and ECC and ECC and FSC. However, a negative and non significant correlation was observed between TVC and ECC.

4.2.3 Correlation between the mean bacterial count of the samples from source C

The correlation coefficient between the mean bacterial count of the carcasses belonging to the source C are shown in Table 12.

Table 12. Correlation coefficient between the mean bacterial count of the carcasses from source C

Bacterial count	Correlation coefficient between mean count			
	TVC	CC	ECC	FSC
TVC		-0.025	-0.184	-0.062
CC			0.165	0.713*
ECC				-0.087

* P <0.05

Analysis of the data revealed a positive and significant (P<0.05) correlation between CC and FSC, but a positive and non significant association

was observed between CC and ECC. However, a negative and non significant correlation exist between TVC and CC, TVC and ECC, TVC and FSC and ECC and FSC.

4.3 Isolation and identification of bacteria

The carcass rinse obtained from 60 chicken carcasses were subjected to the isolation and identification of *Escherichia coli,* salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes.*

4.3.1 *Escherichia coli*

During the investigation *Escherichia coli* was isolated from chicken carcasses belonging to the source A, B and C. The per cent of carcasses which had *Escherichia coli* is shown in Table 13.

Table 13. The per cent of chicken carcasses from which *Escherichia coli* was isolated and identified

Source of carcass	Number of samples	
	Positive	Per cent
A	11	55
B	13	65
C	7	35
Total	31	51.67

N = 20, from each source

Out of the 60 carcasses, 31 (51.67%) had the organism. The organism was isolated from 65 per cent of the carcass belonging to source B, whereas only 35% of the carcass from source C had the organism.

4.3.1.1 Serotyping of *Escherichia coli*

A total of 53 *Escherichia coli* were isolated and identified by cultural, morphological and biochemical tests. All the isolates were subjected to Eijkman test. Only 44 isolates revealed Eijkman test positive (*Escherichia coli* type I) which were serotyped at the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh and the results are shown in Table 14. The nine Eijkman test negative isolates belonged to *Escherichia coli* type III.

Table 14. Distribution of *Escherichia coli* serotypes isolated from the three sources

Serotype	Number of isolate from the source			Total
	A	B	C	
05	2	--	2	4
08	--	1	1	2
014	2	--	--	2
025	--	4	--	4
033	--	1	--	1
041	--	1	--	1
049	--	2	--	2
066	1	--	--	1
078	2	--	--	2
081	3	--	--	3
084	1	--	--	1
085	1	--	--	1
091	1	--	--	1
0116	1	--	--	1
0121	1	2	--	3
0131	--	--	2	2
0132	--	1	--	1
0146	--	1	--	1
0150	--	1	--	1
0157	--	1	--	1
0161	--	--	1	1
Rough	2	3	1	6
Untypable	--	2	--	2

Of the 44 isolates, 36 belonged to 21 serotypes, six were categorized as rough strains and remaining two were untypable.

Out of the 20 isolates from the source B, one was typed as 0157. The other two sources did not reveal the presence of this serotype. The isolates typed from this source belonged to 10 serotypes and the serotypes 025, 033, 041, 049, 0132, 0146, 0150 and 0157 were isolated only from this source.

Of the 17 *Escherichia coli* isolated from the source A, 15 fell into 10 serotypes. Of this, eight serotypes viz. 014, 066, 078,081, 084, 085, 091 and 0116 were isolated only from this source.

Of the seven isolates from source C, six were serotyped and belonged to four serotypes. The serotype 0131 and 0161 were unique from this source.

4.3.2 *Salmonella*

The 60 chicken carcasses were analysed for the detection of salmonellae. Two isolates were identified as salmonellae based on the cultural, morphological, staining reaction and biochemical characteristics and are given in Table 15.

Table 15. Biochemical reactions shown by two salmonella isolates

Characteristics	Isolates	
	1	2
Primary tests		
Gram's reaction	-	-
Catalase	+	+
Oxidase	-	-
OF	F	F
Motility	NM	NM

Secondary tests		
TSI	Acid butt, alkali slant, no H_2S	Acid butt, alkali slant, no H_2S
Lysine decarboxylase	+	+
Phenylalanine deaminase	-	-
ONPG	-	-
Urease	-	-
Indole	+	+
MR	+	+
VP	-	-
Citrate	-	-
Carbohydrate utilization		
Glucose	+	+
Mannitol	+	+
Maltose	+	+
Lactose	-	-
Sucrose	-	-
Salicin	-	-

F = fermentation, NM = non motile, - = negative reaction, + =positive reaction

4.3.3 Staphylococci

The 60 chicken carcasses were tested for the isolation of staphylococci. Only two (10%), seven (35%) and nine (45%) carcasses belonging to source A, B and C revealed the presence of the organism. All isolates revealed the cultural, morphological and biochemical reactions described by Barrow and Feltham (1993) and were coagulase negative.

4.3.4 Listeria monocytogenes

Listeria monocytogenes was not detected in any of the chicken carcasses.

Discussion

5. DISCUSSION

A total of 60 chicken carcasses, 20 each, from three different sources viz., A, B and C were evaluated for their bacterial quality by estimating the total viable count, coliforms count, *Escherichia coli* count and faecal streptococcal count. The samples were also screened for the presence of *Escherichia coli*, salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes*.

Total viable count

Analysis of variance test of the data revealed significant ($P<0.05$) difference between the mean count of the samples from source B and C. The overall mean count, 7.89 ± 0.07 $\log_{10}$ cfu/ml, of the carcasses was one log greater than that reported by Vorster *et al.* (1994) and two logs greater than that obtained by Bailey *et al.* (2000) and Berrang *et al.* (2000).

One of the samples from source B had count as high as 10^9 cfu/ml of the carcass rinse, whereas only three samples from source C had count as low as 10^6 cfu/ml. The count at the latter level was observed in 96 per cent of chicken samples obtained from the local market (Nair *et al.*, 1990). The count at the level of 10^8 cfu/ml of the carcass rinse was observed in 33.33 per cent of the samples. The meat stored at chill temperatures and showing evidence of spoilage has count at the above level (Rao *et al.*, 1998). Thus, the presence of organism at the above level in the freshly prepared

carcass reduces its shelf life. However, the count in 55 per cent samples each from source A and B and 60 per cent samples from source C was at the level of 10^7 cfu/ml of carcass rinse.

The high count of the broiler chicken carcasses belonging to the three sources could be attributed to the contamination of the carcasses from the wooden block on which all dressing process of the chicken carcass was carried out. Bacterial contamination of the carcasses during dressing of the chicken were inherent since the skin of the carcass was removed with dry feathers (Stern *et al.*, 1995) and also due to handling and processing of carcasses (Murugkar *et al.*, 1993). The above observation indicate that considerable improvement in the bacterial quality of chicken carcasses can be made by improving the dressing process and the hygienic practices followed during the production of chicken carcass.

Coliforms count

Analysis of variance test of the data revealed significant ($P<0.05$) difference between the count of the carcasses belonging to the source A and C and between B and C. The overall mean coliforms count of 60 chicken carcasses from the three sources was 4.97 ± 0.10 $\log_{10}$ cfu/ml. The count was 2.7 logs higher than that reported by Jetton *et al.* (1992) and Bailey *et al.* (2000) and 1.67 logs higher than that observed by Berrang *et al.* (2000). The mean count of the samples from all the three source of the present investigation was also higher than that reported by the above authors.

Of the 60 carcass rinse, three samples had count at the level of 10^6 cfu/ml, whereas only one sample from source C had count at the level of 10^2 cfu/ml. The count in 50 per cent of the samples was at the level of 10^5 cfu/ml and that in 31.67 per cent of the samples was at the level of 10^4 cfu/ml. Janky *et al.* (1978) reported a mean count at the latter level in carcass rinse.

Coliforms may be faecal or non-faecal in origin. The organisms are generally accepted as an index of faecal pollution. The count in such a high level indicate that the sanitary practices followed during the slaughter and dressing of poultry was not satisfactory. The level of organism in the carcasses might be attributed to the contamination of the carcasses from the intestinal content.

***Escherichia coli* count**

Analysis of variance test of the data revealed significant ($p<0.05$) difference between mean ECC of samples from source A and C and B and C. The organism could not be detected from 65 per cent of the samples from source C and the mean count in carcass rinse from this source was as low as 1.08 ± 0.35 $\log_{10}$ cfu/ml. The count observed in the present study from source C was much lower as compared to 1.53 $\log_{10}$ cfu/ml recorded by Jetton *et al.* (1992). The mean ECC of the samples belonging to source A and B were almost similar to the count observed by Vorster *et al.* (1994) and Berrang *et al.* (2000). *Escherichia coli* count at the level of 10^4 cfu/ml of the carcass rinse

was detected in 26.67% of the samples, whereas only 3.33 per cent of the sample had count at the level of 10^2 cfu/ml. However, 20 per cent of the samples had count at the level of 10^3 cfu/ml.

The high count of the organism in the carcasses could be attributed to the high *E. coli* count is the intestinal tracts of broiler chicken (Vorster *et al.*, 1994) and also due to careless evisceration (Pattnaik *et al.*, 1997). That the contamination of the carcasses from the source C was found to be the least could be due to the satisfactory techniques followed during evisceration.

Faecal Streptococcal Count

A highly significant ($P<0.01$) difference between the mean faecal streptococcal count of the samples of the source A and B, A and C and B and C was observed. The overall mean count of the samples was 4.32 ± 0.09 $\log_{10}$ cfu/ml of the carcass rinse. The samples belonging to the source C had the lowest count. The lower count of the samples of this source may be attributed to the better evisceration practices followed during its production.

The FSC was found to vary between 10^3 and 10^5 cfu/ml of the carcass rinse. The count at the latter level was observed only in nine (15%) carcasses belonging to the source A. The count in 28 (21.4%) samples was at the level of 10^4 cfu/ml. The organisms are primarily of faecal origin and hence generally regarded as indicators of food sanitary quality. The very high count

and the detection of the organism in all samples indicate unhygienic practices and poor sanitary measures followed during the dressing of chicken.

Correlation between bacterial counts

A positive and significant ($P<0.05$) correlation existed between overall mean CC and FSC. A similar association was observed between the mean CC and FSC of the samples from source B and C and also between the mean TVC and FSC of the samples from source A. These findings suggest that an increase in FSC on the carcass surface lead to a corresponding increase in CC. Faecal streptococci are of intestinal origin and an increase in the organism with a corresponding increase in CC indicate that majority of the coliform organisms are of intestinal origin. This phenomenon was also found in the samples obtained from source B and C. However, such an association was not observed in the samples obtained from source A. Therefore, the contamination of the carcasses might have occurred from the faecal matter during dressing or from the wood on which all operations of dressing was carried out, from the knife used for dressing, the hands of the personnel engaged in the dressing process or all of them might have contributed to the contamination of the carcasses. Such contaminants reduce the shelf life of carcasses.

Escherichia coli

Escherichia coli is an indicator and pathogenic organism found both in man and animals excreta and its presence in carcasses indicate the contamination of the carcasses with the excreta and/or with the contaminated

environmental source like water. Thus, the presence of the organism in the meat indicate unsatisfactory hygienic practices followed during the dressing of poultry.

In the present study, *Escherichia coli* was isolated from 51.67 per cent of the carcasses. The isolation of the organism from 79.1 per cent (Vorster *et al.*, 1994) and 60.87 per cent of samples (Sharma *et al.*, 1995a) indicate that the rate of isolation of the organism from the samples of the present study was much lower than that observed by them. The organism was detected in 65 per cent of the samples from the source B and from 55 per cent and 35 per cent of samples belonging to the source A and C respectively.

Thirty six of the 44 isolates were grouped under 21 serotypes, six strains were rough and two untypable. Of the 21 serotypes, 12 (57.13%) were found to be pathogenic which include 05, 08, 025, 078, 084, 085, 091, 0116, 0121, 0132, 0146 and 0157.

The serotypes 08, 025, 078 and 085 were categorized as Enterotoxigenic *Escherichia coli* (ETEC) which are associated with infantile and traveller's diarrhoea (WHO scientific working group, 1981). Black *et al.* (1981) reported the isolation of the serotype 078 from diarrhoeal patients who had contact (10%) with either water samples or animals positive for ETEC. The serotype 078 was associated with egg peritonitis (Panneerselvam *et al.*, 1988) and colisepticemia and enteritis in poultry (Reddy *et al.*, 1994). The

serotype was also isolated from diarrhoea in calves (Blanco *et al.*, 1988) and market poultry meat samples (Nair *et al.*, 1990).

The ETEC serotype 08 was isolated from one sample each from the source B and C. The serotype could be isolated from poultry with colisepticemia (Panneerselvam *et al.*, 1988) and enteritis (Sharada *et al.*, 1999). The organism was also isolated from diarrhoeic calves (Sherwood *et al.*, 1985) and diarrhoeic and healthy pigs (Garabal *et al.*, 1996).

The serotype 025 was isolated from four samples from source B. The serotype was isolated from cases of peritonitis in poultry (Sharada *et al.*,1999).

The serotype 085 was isolated from one of sample belonging to the source A. Samples from the other sources were found free from this organism. The serotype was isolated from poultry with colisepticemia (Panneerselvam *et al.*, 1988).

Enterohaemorrhagic *Escherichia coli* (EHEC) or the Verotoxigenic *Escherichia coli* (VTEC) organisms isolated from the samples included serotypes 05, 084, 091, 0116, 0121, 0132, 0146 and 0157. These strains can produce a toxin similar to that produced by *Shigella dysentriae* and hence also called Shiga like toxin producing *Escherichia coli.* Samadpour *et al.* (1994) reported the isolation of Shiga like toxin producing *Escherichia coli* from 12 per cent of chicken samples, 23 per cent of beef samples, 18 per cent pork samples, 48 per cent lamb samples and 63 per cent of veal samples.

The serotype 0157 was isolated from diarrhoeic calves (Blanco *et al.*, 1988) and also from oedema disease in pigs (Garabal *et al.*, 1996). Isolation of serotype 05 was made from samples belonging to source A and C. The isolation of this serotype was reported from chicken meat (Nair *et al.*, 1990 and Sharma *et al.*, 1995a) and also from poultry with pericarditis and perihepatitis (Sharada *et al.*, 1999).

The serotypes 084, 091 and 0116 were isolated from one sample each from source A. Serotypes 091 and 0116 have been isolated from poultry with colisepticaemia, enteritis and omphalitis (Reddy *et al.*, 1994). However, the isolation of serotype 084 from buffalo beef (Banerjee *et al.*,2001) and 091 from pork (Samadpour *et al.*, 1994) was also reported.

The isolation of ETEC and EHEC from poultry carcass rinses in the present study indicate the importance of poultry meat in causing food poisoning outbreaks. Most of the serotypes obtained are linked either to poultry meat or to some pathological conditions in poultry which indicate that these birds can harbour the organism and may act as a potential source of infection to human beings. The serotype 0157 is considered as a food borne pathogen of growing importance as it has been linked with several food borne outbreaks. According to Reilly (1998) faecal contamination of water and other foods and cross contamination during food preparation are important routes of infection. Person to person spread and direct contact with farm animals and birds carrying the organism is also a recognized source of infection. Moreover, it has been found that chicks can readily be colonized with 0157 VTEC and continue to be

long term shedders of the organism (Heuvelink *et al.*, 1999) contributing to the chances of increase in food born diseases from chicken meat.

Salmonella

Salmonella was isolated from two (10%) of the chicken carcass rinses obtained from source B, but the organism was not detected from carcass rinse of the samples belonging to the source A and C. The per cent of isolation of the organism in the present study was lower as compared to that from broiler carcasses (Rusui *et al.*, 1996) and from raw whole chicken (Harrison *et al.*, 2001). However, Turtura (1991) could not detect the organism from poultry carcass rinses. As per the government of India Standards for raw meat (chilled/frozen) *Salmonella* should be absent in all the five samples examined (Rao *et al.*, 1998). According to The Food Act, Government of Mauritius (1998) *Salmonella* should be absent in 25 g of raw meat and poultry.

The primary habitat of *Salmonella* spp is the intestinal tract of animals and birds. Hence, these organisms get excreted in their faeces. Poultry is an important reservoir of the organism and hence egg, chicken and its products constitute the most important vehicle of food borne salmonellosis. Thus, an increase in the incidence of salmonellosis in poultry increases the disease is human beings (Zivkovic *et al.*, 1997).

Staphylococci

Staphylococci were isolated from 18 (30%) of the 60 chicken carcass rinses. The organism has been isolated from 10, 35 and 45 per cent of

the samples from the sources A, B and C respectively. However, none of the isolates were coagulase positive. Though the organisms are present on the skin of slaughtered poultry carcasses, the common source of contamination is the food handlers (Bremner, 1977). The knives and chopping blocks and also the unsanitary habits of the retailers causes contamination of the carcasses (Nkanga and Uraih, 1981). The organism is ubiquitous in nature and hence their presence especially the non-coagulase positive organism in the carcass rinse is an indication of poor hygienic practices followed during the production of carcasses. Coagulase production by the organism is as indicator of their ability to produce enterotoxins, but its production by coagulase negative organisms is also reported (Jay, 1996). Therefore, the presence of coagulase negative organisms may also be considered as public health significant.

Listeria monocytogenes

All chicken carcasses were found free of *Listeria monocytogenes*.

Summary

6. SUMMARY

During the investigation, a total of 60 broiler carcasses, consisting of 20 each, from stalls selling dressed birds brought from outside the state (A), stalls selling dressed birds which are reared locally (B) and dressed birds obtained from the Kerala Agricultural University Poultry Farm (C) were collected and evaluated for their bacterial quality by estimating total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC). The samples were also subjected to isolation and identification of food borne pathogens such as *Escherichia coli*, salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes*.

All bacterial count were subjected to statistical analysis. Analysis of variance test revealed significant ($P<0.05$) difference between the mean total viable count of samples from source B and C. The carcass rinse had an overall mean count of 7.89 ± 0.07 $\log_{10}$ cfu/ml. The samples belonging to the source B had the highest mean count (8.06 ± 0.11 $\log_{10}$ cfu/ml). The mean count of the carcass rinse of the samples from source A and C was 7.94 ± 0.08 and 7.69 ± 0.13 $\log_{10}$ cfu/ml, respectively.

One of the samples belonging to the source B had count at the level of 10^9 cfu/ml of carcass rinse, whereas three samples from source C had count at the level of 10^6 cfu/ml. Of the 60 carcasses, 56.67 per cent had count at the level of 10^7 cfu/ml and the count in 36.67 per cent was at the level of 10^8 cfu/ml.

Analysis of variance test of the data revealed significant ($P<0.05$) difference between the mean coliforms count of the samples obtained from the source A and C and B and C. The samples obtained from source A had the highest mean count (5.30 ± 0.10 $\log_{10}$ cfu/ml). The lowest mean count was observed in samples obtained from source C (4.37 ± 0.17 $\log_{10}$ cfu/ml). The mean count of carcass rinse from source B was 5.23 ± 0.13 $\log_{10}$ cfu/ml.

The distribution of coliforms in the carcass vary from 10^2 to 10^6 cfu/ml of carcass rinse. One of the samples from source C had count at the level of 10^2 cfu/ml. The count in one of the samples from source A and two samples in source B was at the level of 10^6 cfu/ml. The count in 35 per cent samples belonging to the source C was at the level of 10^3 cfu/ml. Of the 60 samples analysed, 50 per cent had count at the level of 10^5 cfu/ml and the count in 31.67 per cent of the samples was at the level of 10^4 cfu/ml.

Analysis of variance test of ECC of the samples revealed significant ($P<0.05$) difference between mean count of the samples from source A and C and B and C. The presence of the organism was detected in 51.67 per cent of the samples. The overall mean count of the samples was 2.20 ± 0.27 $\log_{10}$ cfu/ml. The samples belonging to the source C had the lowest mean count (1.08 ± 0.35 $\log_{10}$ cfu/ml). The mean count of the carcass rinses from source A and B was 2.83 ± 0.48 and 2.66 ± 0.46 $\log_{10}$ cfu/ml, respectively.

The distribution of ECC of the samples ranged between 10^2 and 10^4 cfu/ml of the carcass rinse. The count at the former level was observed in

five per cent of the samples belonging to the source B and 10 per cent of samples obtained from source C. The count in 26.67 per cent samples was at the level of 10^4 cfu/ml and in 20 per cent samples the count was at the level of 10^3 cfu/ml.

Analysis of variance test of the FSC of the samples revealed highly significant ($P<0.01$) difference between mean count of the samples belonging to the source A and B, A and C and B and C. The overall mean count of the samples was 4.32 ± 0.09 $\log_{10}$ cfu/ml of the carcass rinse. The highest mean count was observed in the samples belonging to the source A (4.99 ± 0.10 $\log_{10}$ cfu/ml) and the lowest mean count in the samples collected from the source C (3.75 ± 0.10 $\log_{10}$ cfu/ml). The mean count of the samples from source B was 4.20 ± 0.10 $\log_{10}$ cfu/ml).

The distribution of faecal streptococcal count vary from 10^3 to 10^5 cfu/ml. The count at the latter level was observed in 45% of the carcasses from the source A. The count in 46.67 per cent of the samples was at the level of 10^4 cfu/ml of carcass rinse and in 38.33 per cent samples had the count at the level of 10^3 cfu/ml.

The correlation coefficient between the various count were determined. A positive and significant ($P<0.05$) correlation was observed between the overall mean CC and FSC. The association between overall mean TVC and CC, TVC and ECC, TVC and FSC, CC and ECC and ECC and FSC was also positive, but non-significant.

A significant ($P<0.05$) and positive correlation was observed between TVC and FSC of the samples belonging to the source A. A positive but non-significant association existed between TVC and CC, TVC and ECC, CC and ECC and CC and FSC. A negative and non-significant correlation was observed between ECC and FSC.

A significant ($P<0.05$) and positive correlation was observed between CC and FSC of the samples belonging to the source B. However, the association between TVC and CC, TVC and FSC, CC and ECC and ECC and FSC of the samples from this source was positive but non-significant. The correlation between TVC and ECC was negative and non-significant.

The association between the mean CC and FSC of the samples belonging to the source C had a significant ($P<0.05$) and positive correlation. A positive and non-significant correlation existed between CC and ECC, whereas a negative and non-significant association was observed between TVC and CC, TVC and ECC, TVC and FSC and ECC and FSC.

Escherichia coli was isolated from 31 (51.67%) samples. From these samples, 44 *Escherichia coli* isolates were obtained and were serotyped at the National Salmonella and Escherichia Centre (NSEC), Central Research Institute, Kasauli, Himachal Pradesh. The isolates fell into 21 serotypes, six rough strains and two untypables. The 21 serotypes consisted of 05, 08, 014, 025, 033, 041, 049, 066, 078, 081, 084, 085, 091, 0116, 0121, 0131, 0132, 0146, 0150, 0157 and 0161. The serotypes 014, 066, 078, 081, 084, 085, 091

and 0116 were isolated only from the samples belonging to the source A. Only the samples from source B revealed the presence of the serotypes 025, 033, 041, 049, 0132, 0146, 0150 and 0157. Serotype 0131 and 061 were isolated from samples from source C. The serotypes 08, 025, 078 and 085 were categorized as Enterotoxigenic *Escherichia coli* (ETEC) and 05, 084, 091, 0116, 0121, 0132, 0146 and 0157 as Enterohaemorrhagic *Escherichia coli* (EHEC).

Only two salmonellae were isolated from the samples belonging to the source B, but none of the samples from the source A and C revealed the presence of this organism.

Staphylococci were isolated from 30 per cent of the carcasses, but none of these isolates were coagulase positive.

Listeria monocytogenes was not isolated from any of the carcasses tested.

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BACTERIA OF PUBLIC HEALTH SIGNIFICANCE IN BROILER DRESSED CHICKEN

By

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ABSTRACT OF THE THESIS

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ABSTRACT

In the present investigation, 60 chicken carcasses, consisting of 20 each, collected from retail shops selling dressed birds which were brought from outside the state (A), shops selling locally reared dressed birds (B) and dressed chicken from the Kerala Agricultural University Poultry Farm (C). The bacterial quality of each carcass was evaluated by estimating the total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC). All samples were also tested for the presence of bacterial pathogens such as *Escherichia coli*, salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes*.

Analysis of variance test of the mean TVC revealed significant ($P<0.05$) difference between the count of the samples from source B and C. The overall mean TVC of the samples was 7.89 ± 0.07 $\log_{10}$ cfu/ml of the carcass rinse. The samples from source B had the highest mean TVC (8.06 ± 0.11 $\log_{10}$ cfu/ml) and lowest mean count was seen in the samples belonging to the source C (7.69 ± 0.13 $\log_{10}$ cfu/ml). The samples from source A had a mean TVC of 7.94 ± 0.08 $\log_{10}$ cfu/ml. The count at the level of 10^9 cfu/ml was observed in one of the samples belonging to the source B. The count in three (5%), 34 (56.67%) and 22 (36.67%) samples was at the level of 10^6, 10^7 and 10^8 cfu/ml, respectively.

Analysis of variance test showed significant ($P<0.05$) difference between the mean CC of the samples from source A and C and B and C. The overall mean CC of the carcass rinse was 4.97 ± 0.10 $\log_{10}$ cfu/ml. The samples from the source A had the highest mean count (5.30 ± 0.10 $\log_{10}$ cfu/ml) and lowest in the samples from source C (4.37 ± 0.17 $\log_{10}$ cfu/ml). The mean CC of samples from source B was 5.23 ± 0.13 $\log_{10}$ cfu/ml. The count in 1.67 per cent and five per cent of the samples was at the level of 10^2 and 10^6 cfu/ml, respectively. The count in 11.67, 31.67 and 50 per cent samples was at the level of 10^3, 10^4 and 10^5 cfu/ml, respectively.

Analysis of variance test revealed significant ($P<0.05$) difference between mean ECC of the samples belonging to the source A and C and B and C. The overall mean ECC of samples from the three sources was 2.20 ± 0.27 $\log_{10}$ cfu/ml. The mean count of the samples belonging to the source A, B and C was 2.83 ± 0.48, 2.66 ± 0.46, 1.08 ± 0.35 $\log_{10}$ cfu/ml, respectively. The count in five, 20 and 26.67 per cent of the carcasses was at the level of 10^2, 10^3 and 10^4 cfu/ml, respectively.

Analysis of variance test revealed highly significant ($P<0.01$) difference between FSC of samples from source A and B, A and C and B and C. The overall mean FSC of the samples was 4.32 ± 0.09 $\log_{10}$ cfu/ml. The highest mean count was observed in samples from source A (4.99 ± 0.10 $\log_{10}$ cfu/ml) and the lowest mean count in samples from source C (3.75 ± 0.10 $\log_{10}$ cfu/ml). The mean FSC in samples from source B was 4.20 ± 0.10 $\log_{10}$ cfu/ml.

The count in 38.33, 46.67 and 15 per cent samples were at the level of 10^3, 10^4 and 10^5 cfu/ml, respectively.

Correlation coefficient test of the data revealed significant ($P<0.05$) association between the mean CC and FSC. A positive and non-significant correlation existed between mean TVC and CC, TVC and ECC, TVC and FSC, CC and ECC and ECC and FSC.

Escherichia coli was isolated from 51.67% of the samples. The 44 *E. coli* isolated from the samples were serotyped and fell into 21 serotypes, six rough strains and two untypables. The serotypes consisted of 05,08, 014, 025, 033,041, 049, 066,078, 081, 084, 085, 091, 0116, 0121, 0131, 0132, 0146, 0150, 0157 and 0161. Salmonellae were isolated from two samples. Staphylococci were isolated from 30% of the samples, but none of them were coagulase positive. All samples were found free from *Listeria monocytogenes.*