172253

EVALUATION OF BACTERIOLOGICAL QUALITY OF PROCESSED CHICKEN

RAJI ROSE JACOB

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

2003

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis entitled **"EVALUATION OF BACTERIOLOGICAL QUALITY OF PROCESSED 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
6-11-2003

RAJI ROSE JACOB

CERTIFICATE

Certified that the thesis entitled **"EVALUATION OF BACTERIOLOGICAL QUALITY OF PROCESSED CHICKEN"** is a record of research work done independently by **Dr. Raji Rose Jacob**, 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.

Dr. E. Nanu
(Chairman, Advisory Committee)
Professor and Head
Department of Veterinary Public Health
College of Veterinary and
Animal Sciences, Mannuthy

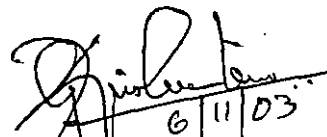
Mannuthy

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Raji Rose Jacob**, a candidate for the degree of Master of Veterinary Science in Veterinary Public Health, agree that the thesis entitled **"EVALUATION OF BACTERIOLOGICAL QUALITY OF PROCESSED CHICKEN"** may be submitted by Dr. Raji Rose Jacob, in partial fulfilment of the requirement for the degree.

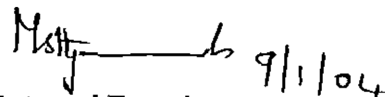
Dr. E. Nanu
(Chairman, Advisory Committee)
Professor and Head
Department of Veterinary Public Health
College of Veterinary and Animal Sciences, Mannuthy

Dr. C. Latha
Assistant Professor
Department of Veterinary Public Health
College of Veterinary &
Animal Sciences, Mannuthy
(Member)



Dr. G. Krishnan Nair
Associate Professor
Department of Microbiology
College of Veterinary &
Animal Sciences, Mannuthy
(Member)

Dr. M. Mini
Assistant Professor
Department of Microbiology
College of Veterinary &
Animal Sciences, Mannuthy
(Member)



External Examiner
(Dr. M. Sathyanarayana Rao)

ACKNOWLEDGEMENTS

"My grace is sufficient for you"

(2 Cor. 12:9)

*It gives me inexplicably immense pleasure to place on record the deep sense of gratitude to my mentor and major advisor, **Dr. E. Nanu**, Professor and Head, Department of Veterinary Public Health and Chairman of Advisory Committee for his able guidance, affection, meticulous supervision, creative suggestions and critical comments. It is a matter of great fortune and pride, I deem it, to get an opportunity to work under the potential guidance of an affectionate,dynamic and versatile academician of his calibre and acumen.*

*I am very much grateful to **Dr. C. Latha**, Assistant Professor, Department of Veterinary Public Health and member of Advisory Committee, for the precious timely suggestions and valuable guidance.*

*Words fail to express my gratitude to **Dr. M. Mini**, Assistant Professor, Department of Microbiology and member of Advisory Committee for her valuable and timely advice, understanding, concrete suggestions and constant support throughout the study period.*

*I am obliged to **Dr. G. Krishnan Nair**, Associate Professor, Department of Microbiology and member of Advisory Committee, for his valuable suggestions and critical corrections.*

*I would like to place on record my acknowledgement to **Dr. B. Sunil**, Assistant Professor, Department of Veterinary Public Health for his constructive criticisms, constant support and understanding.*

*I extend my heart-felt thanks to **Mrs. Sujatha**, Assistant Professor and Head, Department of Statistics, for her scholarly help in carry out the statistical analysis of the study.*

I am grateful to the Kerala Agricultural University for providing me the fellowship.

I am extremely thankful to ***Dr. H.G. Brahmne****, Joint Director and Head, National Salmonella and Escherichia Centre, Centre Research Institute, Kasauli for serotyping the E. coli and Salmonella isolates and sending the results on time.*

I extend my thanks to Dean, College of Veterinary and Animal Sciences, Mannuthy and Associate Dean, College of Veterinary and Animal Sciences, Pookot for the facilities provided for this research work.

Words are inadequate to express my deep felt indebtedness to ***Dr. Ani. S. Das, Dr. Saji Eassow, Dr. Sathu, T., Mr. James Mathew*** *and all the processing line workers at MPI, Koothattukulam for their concern and co-operation, which helped to bring out my work to fruitful completion. Let me also reckon my sincere gratitude to* ***Mr. Thomas*** *for providing the necessary transportation facilities to Koothattukulam.*

I greatly acknowledge the co-operation and help rendered to me by the non-teaching staff of Department of Veterinary Public Health, ***Mr. Sundaran, Miss. Sandhya, Mrs. Deepa*** *and* ***Mrs. Thara.***

I am thankful to M/s Peagles, Mannuthy and Rema Nair for the timely assistance in the preparation of this thesis.

The invaluable help extended by librarian and staff of College library, Central library, AIRS Cell and Central Institute of Fisheries Technology are deeply acknowledged.

I owe my sincere gratitude to ***Dr. M. Satyanarayana Rao*** *for his blessings and encouragement.*

I express my gratitude to ***Dr. Aneesh Raj, Dr. Binsy Mathew, Dr. Vrinda Menon, Dr. Bindu Raj*** *and* ***Dr. Ambili, V.S.*** *for their help extended in varied forms.*

My grateful thanks are due to ***Dr. A.M. Chandrasekharan****, Department of Pharmacology for the timely help.*

I do express my very special and sincere thanks to ***Shantha auntie, Rekha*** *and* ***Arun*** *for their love, cordiality and concern.*

Let me thank whole heartedly to my friends, ***Dr. Neena Simon, Dr. Leeba, Dr. Lydia, Dr. Shibi, Dr. Uma*** *and all other colleagues of mine.*

The support given by my colleagues ***Dr. Shiny*** *and* ***Dr. Sethulekshmi*** *throughout my study can not be expressed in words or deeds. And I take this opportunity to extend my very sincere thanks to them.*

I thank the Almighty for having given me very understanding friends ***Dr. Thushara*** *and* ***Dr. Durgga*** *their sisterly affection and sustained encouragement boosted me. Their care and concern helped me to overcome the hardships faced during the period of study and research work.*

I thankfully appreciate all the concern and blissful support bestowed upon by my kindred and in-laws.

I express my heartfelt gratitude to my beloved husband ***Mr. Rojy*** *for his understanding, love , mental support and constant encouragement.*

Words can not express my feelings to ***Achachan, Mummy, Rejani*** *and* ***Rani*** *for their unfailing love, patience, mental support, incessant encouragement, heartfelt blessings and prayers.*

I take this opportunity to thank all who directly or indirectly helped me in fulfilling this endeavour.

Above all, I bow to the ***Almighty*** *for his grace in the successful completion of this course.*

RAJI ROSE JACOB

CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	3
3	MATERIALS AND METHODS	30
4	RESULTS	50
5	DISCUSSION	67
6	SUMMARY	75
	REFERENCES	79
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1.	Mean total viable count of carcasses taken after the removal of head and feet and after evisceration	50
2.	Distribution of chicken carcasses based on total viable count	51
3.	Mean coliforms count of carcasses taken after the removal of head and feet and after evisceration	53
4.	Distribution of chicken carcasses based on coliforms count	53
5.	Mean *Escherichia coli* count of carcasses taken after the removal of head and feet and after evisceration	55
6.	Distribution of chicken carcasses based on *Escherichia coli* count	57
7.	Mean faecal streptococcal count of carcasses taken after the removal of head and feet and after evisceration	57
8.	Distribution of chicken carcasses based on faecal streptococcal count	58
9.	Correlation coefficient between the mean bacterial count of chicken carcass samples collected after the removal of head and feet	60
10.	Correlation coefficient between the mean bacterial count of chicken carcasses samples collected after evisceration	60
11.	The per cent of carcasses yielded *Escherichia coli*	61
12.	Distribution of *Escherichia coli* serotypes	62
13.	*Salmonella* isolated from chicken carcasses	63
14.	*Staphylococcus aureus* isolated from chicken carcasses	64
15.	Mean total viable count of air samples from three different sites on the processing line	65
16.	The mean bacterial load of various processing equipments and water samples	66

LIST OF FIGURES

Figure No.	Title	Page No.
1.	Distribution of chicken carcasses based on total viable count	52
2.	Distribution of chicken carcasses based on coliforms count	54
3.	Distribution of chicken carcasses based on *Escherichia coli* count	56
4.	Distribution of chicken carcasses based on faecal streptococcal count	59

LIST OF FLOW CHARTS

Flow chart No.	Title	Page No.
1.	Isolation and identification of *Escherichia coli* from chicken carcass	34
2.	Isolation and Identification of *Salmonella* from chicken carcass	36
3.	Isolation and identification of *Staphylococcus aureus* from chicken carcass	38
4.	Isolation and identification of *L. monocytogenes* from chicken carcass	39

Introduction

1. INTRODUCTION

Meat is an important livestock product and is being consumed by human being from time immemorial. It has a high biological value in human nutrition but it is a highly perishable commodity. During the year 1980 the country had produced 0.85million metric tonnes of meat. In 1990 and 2000 the meat production in the country was 3.7 and 4.7 million metric tonnes, respectively. This indicate that though the country has the highest livestock population in the world, the quantity of meat produced is too low but an increasing trend in the meat production has been observed during the last 20 years. The per capita meat consumption of the country is only 9.5 g/day which is much less than the recommendation given by the Indian Council of Medical Research (34 g/day).

The poultry population of the country is about three per cent of the world poultry population and has ranked on 21st in the world chicken meat production. The total poultry meat production in the country is eight lakh tonnes and depends on 413 million chicken. The per capita availability of poultry meat in the state is 1.51 kg/year.

In India meat is produced from 3600 slaughter houses which are under the control of local bodies. However a vast majority of these slaughter houses do not have infrastructure for the slaughter and dressing of poultry. The country also have 24 meat processing plants. Most of these plants have poultry processing facility but the infrastructure and hygienic practices of these plants are not satisfactory.

During the slaughter and dressing of poultry, the carcasses get contaminated from various sources. The safety and hygienic quality of the carcasses are largely determined by the presence of microorganisms which are ubiquitous in nature. Bacterial load of dressed chicken invariably depends upon the sanitary practices in the slaughter house, the hygienic condition of the

equipments and environmental condition of the slaughter and dressing line and/or the area.

Bacterial contaminants of the carcasses include both saprophytic and pathogenic organisms. The growth and multiplication of the former group of organisms lead to spoilage of the valuable meat as well as economic loss to meat trade and also loss of valuable food. However, the growth and multiplication of latter group of organisms do not produce much appreciable changes on the carcasses but are associated with food-borne infections and/or intoxication to the consumers. Therefore, frequent monitoring of the carcasses in the dressing line is of great public health significance. Further, on perusal of literature, it is observed that not much studies have been done on the bacterial quality of chicken carcasses in the dressing line. Considering all the above facts the present study was undertaken with the following objectives.

I. To evaluate the bacterial quality of chicken carcasses obtained from the dressing line by estimating.

   a) Total viable count

   b) Coliform count

   c) *Escherichia coli* count

   d) Faecal streptococcal count

II. To detect the presence of bacteria of public health significance

   a) *Escherichia coli*

   b) Salmonellae

   c) *Staphylococcus aureus*

   d) *Listeria monocytogenes*

III. To assess the role of environment and equipments on the contamination of chicken carcasses.

Review of Literature

2. REVIEW OF LITERATURE

2.1 BACTERIAL COUNT

2.1.1 Total Viable Count

Panda (1971) evaluated the incidence and distribution of various types of microorganisms associated with dressed chicken retailed in different parts of the country. The study revealed that the samples collected from Bangalore had total aerobic count varying from 2.5×10^5 to 3.3×10^6 and the corresponding count of the samples collected from Mysore ranged between 5.4×10^6 and 3.90×10^6.

Patterson (1972) estimated the levels of microbial contamination on processed poultry carcasses by different sampling methods. He reported that the samples collected by swabbing had a mean $\log_{10}$ number viable microorganisms of 2.42 ± 0.035 /cm^2 and the samples collected by cutting had the count at the level of 2.68 ± 0.0356 $\log_{10}$/cm^2. However, the samples collected by swabbing and cutting had a mean count of 2.96 ± 0.0373 $\log_{10}$/cm^2.

Maxcy *et al.* (1973) studied the microbial quality of hand deboned fresh ground poultry meat and reported that the samples had total aerobic count at the level of 10^3 to 10^7/g

Janky *et al.* (1978) compared the physical, sensory and microbiological characteristics of brine-chilled, slush-ice chilled and hot packaged broiler carcasses. They reported that the hot packaged broiler carcasses had a mean aerobic plate count of 1.8×10^5/ ml of carcass rinse.

Sauter *et al.* (1978) evaluated the micro flora in 60 each of fresh and thawed frozen fryers. The former samples had a total aerobic bacterial count of 2.2×10^4 organisms/cm^2 and the latter had a mean count of 2×10^4 organisms/cm^2.

Mercuri and Cox (1979) evaluated the bacterial quality of two each of broiler carcass, frozen chicken potpie, frozen mechanically deboned poultry meat, ground beef and pork sausages. The broiler carcasses had a mean aerobic plate count of 5.8 $\log_{10}$/g of chicken skin.

Lillard (1980) studied the effect of chlorine at the level of 20 and 34 ppm and chlorine dioxide at three and five ppm on the bacterial quality of chilled chicken carcasses. The study revealed that untreated carcasses had a mean total viable count of 4.60 $\log_{10}$/g of breast skin. The count on carcasses treated with 20 and 34 ppm of chlorine was 3.55 and 3.83 $\log_{10}$/g, respectively. Carcasses treated with three and five ppm of chlorine dioxide had the mean count of 3.67 and 3.73 $\log_{10}$/g, respectively.

Anand *et al.* (1987) analysed the incidence of micro flora on 52 freshly dressed broiler chicken carcasses and reported that breast, drumstick and underwings had total viable counts in the range of 18×10^1 to 34×10^3, 10×10^1 to 17×10^2 and 20×10^2 to 84×10^4 cfu/cm^2, respectively.

Anand *et al.* (1989) assessed the microbial quality of chicken carcasses during the various stages of dressing. They reported that the mean total viable count of 10 batches of defeathered chicken carcasses varied from 3.1 to 4.8 $\log_{10}$/cm^2 and the count on the washed carcasses ranged between 2.9 and 5.0 $\log_{10}$/cm^2.

Anand *et al.* (1990) evaluated the microbial profile of culled dressed broiler chicken carcasses and also meat and its products. They observed that the dressed carcasses had a mean total aerobic count of 4.28 $\log_{10}$ cfu/g.

Lillard (1990) assessed the levels of aerobic bacteria and *Enterobacteriaceae* on broiler carcasses at six points during processing. The study revealed that the post pick broiler carcasses had a mean aerobic bacterial count of 7.37 ± 0.57 $\log_{10}$ cfu/carcass while the count on pre-chilled broiler carcasses was 6.46 ± 0.60 $\log_{10}$ cfu/carcass.

Nair *et al.* (1990) examined the bacteriological quality of dressed broilers from the processing unit at Central Food Technological Research Institute (CFTRI), Mysore and the local market. The total plate count from the former source was 10^5/g while the count in 96 per cent of the samples from the latter source was 10^6/g and in four per cent samples the count was at the level of 10^7/g.

Anand *et al.* (1991) studied the microbial quality and shelf life of chicken patties stored at –18°C. The mean aerobic plate count of the fresh deboned meat was 4.3 ± 0.05 log/g.

Fliss *et al.* (1991) analysed the microbiological quality of 30 fresh poultry carcasses obtained from the market and reported that the carcasses had a mean total aerobic micro flora of 5.25 $\log_{10}/cm^2$.

Anand *et al.* (1992) assessed the microbial quality of chicken tandoori during their production and storage. They found that dressed birds before seasoning had a mean aerobic plate count of 5.36 ± 0.04 $\log_{10}$ cfu/g.

James *et al.* (1992) conducted a study to measure the degree of contamination by various types of bacteria on broiler carcasses at pre-evisceration, pre-chill, post-chill and post automatic cut. They observed that the mean aerobic plate count of pre-eviscerated chicken carcasses was 4.05 $\log_{10}$ cfu/carcass, while the count per pre-chilled carcass was 3.39 $\log_{10}$ cfu.

Jetton *et al.* (1992) evaluated the influence of distilled water and 0.85 per cent sodium chloride solution on the recovery of total aerobic bacteria, total coliforms, *Escherichia coli* and salmonellae from chilled broiler carcass halves collected from three trials. The mean aerobic plate count obtained in saline and distilled water rinsate was 3.48 and 3.52 $\log_{10}$ cfu/ml, respectively.

Murugkar *et al.* (1993) investigated the microbiological quality of a total of 108 samples consisting of 18 each of whole dressed chicken, boneless legs,

boneless breast, liver, gizzard and kheema. They recorded that the whole dressed chicken had a mean total viable count of 5.57 log cfu/g.

Whittemore (1993) compared plate count, three tube and modified most probable number procedure for estimating the $\log_{10}$ number of microorganisms on 24 poultry carcasses. The mean aerobic plate count of the samples was 3.55 ± 0.35 $\log_{10}$ cfu/ml of carcass rinse.

Abu-Ruwaida *et al.* (1994) determined the effect of processing procedures and overall environmental and hygienic conditions on the microbial load of carcasses at different locations on a processing line in a modern broiler slaughterhouse. The level of bacterial contamination on chicken carcass was evaluated by a 'neck-skin maceration method'. The mean counts of aerobic bacteria recovered from defeathered chicken carcasses collected on days one and two were 7.0 ± 0.39 and 7.2 ± 0.30 $\log_{10}$ cfu/g, respectively, while the counts on eviscerated spray washed carcasses collected on the same days were 6.0 ± 0.38 and 6.3 ± 0.45 $\log_{10}$ cfu/g, respectively.

Vorster *et al.* (1994) conducted a study to determine the incidence of *Escherichia coli* and *Staphylococcus aureus* in ground beef, broilers and processed meats in Pretoria, South Africa and also identify the relationship between these bacteria and the total bacterial count. The mean aerobic plate count of 43 broiler samples was 6.8 $\log_{10}$ cfu/ml of broiler rinse.

Cason *et al.* (1997) assessed the relationships between numbers of aerobic bacteria and the human enteropathogens, salmonellae and *Campylobacter*, on broiler carcasses sampled at various stages during processing. They reported that the mean aerobic plate count on carcasses recovered from processing lines before chilling was 7.13 ± 0.05 and 7.12 ± 0.06 $\log_{10}$ cfu/carcass in 72 salmonellae negative and 18 salmonellae positive carcasses, respectively.

Pattnaik *et al.* (1997) tested qualitatively and quantitatively the microbial load of chicken carcasses processed at local slaughterhouses of Bhubaneswar

city. They found that the mean total aerobic plate count of 30 samples of leg meat from hot (un-chilled) eviscerated carcasses was 7.93 $\log_{10}$/g of sample.

Mokgatla *et al.* (1998) estimated the total plate count on the neck skin of chicken carcasses collected at bleeding, scalding, plucking, evisceration and chilled packaged products. The mean count of the skin samples collected at plucking and evisceration was 1.58×10^6 and 2.51×10^5/g, respectively.

Bailey *et al.* (2000) examined the effect of different refrigeration and freezer temperatures on the microbiological profile of 50 commercially processed broiler chicken carcasses. The mean mesophilic bacteria count of five carcass halves sampled before being chilled or frozen was 4.62 $\log_{10}$/ml of carcass rinse.

Berrang *et al.* (2000) determined the effects of hot water application after defeathering on the levels of *Campylobacter*, coliform bacteria and *Escherichia coli* on broiler carcasses. The mean total aerobes on defeathered carcasses collected before delayed immersion rescald, delayed spray rescald, immediate immersion rescald, immediate spray rescald were 4.4 ± 0.10, 4.6 ± 0.12, 4.3 ± 0.09 and 4.5 ± 0.09/ml of whole carcass rinse, respectively.

Berrang *et al.* (2001) evaluated the level of *Campylobacter*, coliforms, *Escherichia coli* and total aerobic bacteria from broiler parts with and without skin. They reported that the mean of total aerobes recovered from the skin of breasts, thighs and drumsticks of New York Dressed broiler carcasses were 3.8 ± 0.3, 4.2 ± 0.3 and 4.2 ± 0.3 $\log_{10}$ cfu/g, respectively. The counts on the skin of breast fillets, thighs and drumsticks of eviscerated pre-chilled broilers were 4.4 ± 0.2, 4.6 ± 0.3 and 4.0 ± 0.3 $\log_{10}$ cfu/part, respectively.

Raj (2002) studied bacteria of public health significance in 60 broiler chicken carcasses collected from three different sources. She reported that the overall mean total viable count of the samples was 7.89 ±0.07 $\log_{10}$ cfu/ml of the whole carcass rinse.

Fluckey *et al.* (2003) estimated the numbers of total aerobes, coliforms, psychrotrophic organisms, generic *E. coli*, *Salmonella* spp. and *Campylobacter* spp., on broiler carcasses before and after evisceration and also after chilling. The aerobic plate counts of broiler carcasses before and after evisceration were 4.48 and 3.81 log_{10} cfu/ml of rinse. The count on the carcasses after chilling was 3.23 log_{10} cfu/ml of rinse.

2.1.2 Coliforms Count

Panda (1971) assessed the microbial load on the skin surface of dressed chicken collected from three shops in Bangalore and two shops in Mysore. He reported that the average coli-aerogenes count of samples collected from Bangalore ranged from 78 to 104/cm^2, while the corresponding count of the samples from Mysore ranged between 98 and 116/cm^2.

Maxcy *et al.* (1973) evaluated the coliforms count of 39 samples of fresh hand deboned ground poultry meat and reported that the count ranged from 10^1 to 2×10^4/g.

Janky *et al.* (1978) studied physical, sensory and microbiological characteristics of brine -chilled, slush ice-chilled and hot packaged carcasses. The study revealed that the mean MPN faecal coliforms count of hot packaged birds was 5.2×10^2/ml of carcass rinse.

Mercuri and Cox (1979) estimated the coliforms count of the skin of two broiler chicken carcasses purchased from a local supermarket. The count of one of the samples was 2.0 log/g of broiler skin and the count on the other carcass skin was 1.8 log/g.

Lillard (1980) assessed the effect of chlorine and chlorine dioxide on the bacterial quality of chilled chicken carcasses. The study revealed that untreated chicken breast skin had a mean faecal coliforms count of 3.07 log_{10}/g. The counts on carcasses treated with 20 and 34 ppm of chlorine were 1.39 and 1.168

log_{10}/g, respectively. Carcasses treated with three and five ppm of chlorine dioxide had the mean count of 1.86 and 1.81 log_{10}/g, respectively.

Campbell *et al.* (1983) conducted a survey of nine selected chicken eviscerating plants to determine the levels of coliforms, *Escherichia coli* and salmonellae on eviscerated chicken. The geometric mean of the coliforms recovered from samples collected at the entry of the chiller ranged between five and 130/cm^2.

Cox *et al.* (1983) reported that broiler thigh meat used in the preparation of chicken patty products had a coliforms count >3.4 log_{10}/g.

Anand *et al.* (1987) analysed the micro flora on 52 freshly dressed broiler samples. The non-faecal coliforms count of breast, drumstick and underwings ranged from 0 to 4 x 10^1, 0 to 2 x 10^2 and 0 to 12 x 10^2 cfu/cm^2, respectively.

Anand *et al.* (1989) reported that the average coliforms count of 10 batches of defeathered chicken carcasses ranged from 1.3 to 3.3 log_{10}/cm^2 and the count on washed carcasses from the same batches ranged between zero and 3.1 log_{10}/cm^2.

Anand *et al.* (1990) conducted a critical point analysis for assessing the microbial quality of chicken sausages during their production and storage. They observed that the dressed broilers had a coliforms count of 1.84 log/g.

Nair *et al.* (1990) estimated the coliforms count of 50 each of dressed broiler chicken carcasses obtained from the local market and the poultry processing unit at CFTRI, Mysore. The count of the samples from the former source ranged from 10^3 to 10^5/g and that from the latter source ranged between 10^2 and 10^4/g.

Anand *et al.* (1991) assessed the microbial profile of chicken patties during their preparation and reported that the mean coliforms count of mechanically deboned meat from culled birds was 2.41 ± 0.04 log/g.

Fliss *et al.* (1991) reported that 30 poultry carcasses collected from markets had a mean faecal coliforms count of 1.62 log_{10}/cm^2.

Anand *et al.* (1992) examined the microbial quality of chicken tandoori during their production and storage. They recorded that the dressed birds had a mean coliforms count of 3.05 ± 0.05 log/g.

Jetton *et al.* (1992) evaluated the recovery of total coliforms from chilled broiler carcass halves using different rinse media. The study revealed that mean total coliforms recovered using physiological saline and distilled water, as rinse media were 2.26 and 2.11 log_{10} cfu/ml, respectively.

Mead *et al.* (1993) analysed neck skin samples taken from chicken and turkeys at all main stages of processing and recorded that mean coliforms count of the samples after packaging ranged from 2.7 to 3.8 log_{10} cfu/g.

Murugkar *et al.* (1993) reported that, out of 18 whole chicken carcasses examined, five carcasses revealed the presence of faecal coliforms and the mean count of the organism was 3.62 log cfu/g.

Abu-Ruwaida *et al.* (1994) estimated the coliforms count of chicken carcasses at various stages of processing in a modern commercial slaughterhouse in Kuwait. The mean count on defeathered carcasses obtained on days one and two was 5.4 ± 0.58 and 5.6 ± 0.34 log_{10} cfu/g of carcass neck skin, respectively. The neck skin of the eviscerated and washed carcasses collected on the same days had the count in the range of 5.1 ± 0.62 and 5.2 ± 0.80 log_{10} cfu/g.

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

Bailey *et al.* (2000) assessed the coliforms count of five chicken carcass halves sampled before being chilled or frozen and reported the mean count as 2.25 log_{10}/ml rinse.

Berrang *et al.* (2000) reported that the mean counts of coliforms recovered from the defeathered carcasses collected before delayed immersion rescald, delayed spray rescald, immediate immersion rescald and immediate spray rescald treatments were 3.3 ± 0.14, 3.3 ± 0.16, 2.7 ± 0.21 and 3.2 ± 0.20 $\log_{10}$ cfu/ml of whole carcass rinse, respectively.

Berrang *et al.* (2001) evaluated the bacterial population of broiler parts with and without skin. The mean coliforms counts recovered from the skin of breasts, thighs and drumsticks of New York Dressed broiler carcasses were 2.2 ± 0.3, 2.6 ± 0.4 and 2.4 ± 0.3 $\log_{10}$ cfu/g, respectively. The corresponding counts of the eviscerated prechilled broiler carcasses were 3.1 ± 0.4, 3.2 ± 0.3 and 2.6 ± 0.5 $\log_{10}$ cfu/part, respectively.

Raj (2002) assessed the coliforms count of 60 broiler chicken carcasses obtained from three sources and reported the overall mean count as 4.97 ± 0.10 $\log_{10}$ cfu/ml.

Fluckey *et al.* (2003) assessed the microbial profile of broiler carcasses sampled at various processing points in an air-chill poultry processing plant. They reported that the in-plant coliforms levels were 3.91 $\log_{10}$ cfu/ml of rinse for the before evisceration site and 3.27 $\log_{10}$ cfu/ml of rinse for the after evisceration site.

2.1.3 *Escherichia coli* Count

Campbell *et al.* (1983) conducted a survey to determine the levels of coliforms, *Escherichia coli* and *Salmonella* spp. on eviscerated chicken carcasses obtained from nine chicken eviscerating plants and the data was compared with the findings of another survey conducted 10 years ago. The survey revealed that the geometric mean of *E. coli* counts of the samples collected at the entry of the chiller from plants A, B, C, D, E, F, G, H and J were 1, 2, 3, 3, 4, 22, 2, 5 and 22/cm^2, respectively.

Cox *et al.* (1983) reported that broiler chicken thigh meat used in the preparation of chicken patty products had *E. coli* count at the level of 0.8 to 1.0 log/g.

Nair *et al.* (1990) estimated the *E. coli* count of 50 each of dressed broiler birds collected from the poultry-processing unit of CFTRI, Mysore and the local market. They reported that the samples collected from the processing unit had the count at the level of 10^1/g in one of the samples. The count in 32 and five samples was at the level of 10^2 and 10^3/g, respectively. The count in eight samples from the local market was at the level of 10^2/g. In 34 samples the count was at the level of 10^3/g and in eight samples at the level of 10^4/g.

Fliss *et al.* (1991) reported that 30 poultry carcass samples collected from Tunisian market had a mean *E. coli* count of 0.76 $\log_{10}/cm^2$.

Archile *et al.* (1992) evaluated the chemical and microbiological composition of mechanically deboned poultry meat produced in Zulia state, Venezuela. They reported that the mean MPN *E. coli* count of the samples ranged from 2.97 to 3.35 $\log_{10}$ cfu/g.

James *et al.* (1992) reported the mean *E. coli* count on pre-eviscerated and prechilled carcasses as 2.17 and 1.146 $\log_{10}$ cfu/carcass, respectively.

Jetton *et al.* (1992) evaluated the efficiency of distilled water and physiological saline in recovering the *E. coli* from chilled broiler carcass halves. The mean *E. coli* count recovered with the former rinsate was 1.63 $\log_{10}$ cfu/ml and that with the latter rinsate was 1.53 $\log_{10}$ cfu/ml.

Murugkar *et al.* (1993) studied the microbial profile of poultry products and reported that 18 samples of whole dressed chicken had mean *E. coli* count at the level of 2.89 log cfu/g.

Abu-Ruwaida *et al.* (1994) estimated *E. coli* count on chicken carcasses at various stages of processing in a local plant. They reported that defeathered

carcasses obtained on days one and two had mean count at the level of 4.9 ± 0.63 and 4.8 ± 0.83 log_{10} cfu/g of carcass neck skin, respectively, while the count on eviscerated spray washed carcasses collected on these days was 4.8 ± 0.45 and 4.2 ± 0.53 log_{10} cfu/g of carcass neck skin, respectively.

Vorster *et al.* (1994) reported that the mean MPN count of *E. coli* from 43 fresh South African broilers was 2.4 log_{10}/ml of broiler rinse.

Bailey *et al.* (2000) examined five carcass halves before being chilled or frozen and reported that the samples had a mean *E. coli* count of 1.96 log_{10}/ml of carcass rinse.

Berrang *et al.* (2000) analysed the effects of different types of rescald treatments on the bacterial quality of defeathered carcasses. The study revealed that defeathered carcasses collected before delayed immersion rescald, delayed spray rescald, immediate immersion rescald and immediate spray rescald had mean *E. coli* count at the levels of 2.7 ± 0.16, 3.0 ± 0.13, 2.4 ± 0.25 and 2.9 ± 0.21 log_{10} cfu/ml of rinse, respectively.

Berrang et al (2001) reported that the mean *E. coli* counts from the skin of breasts, thighs and drumsticks of New York Dressed broiler carcasses were 1.9 ± 0.4, 2.3 ± 0.4 and 2.1 ± 0.3 log_{10} cfu/g, respectively. The corresponding counts of the eviscerated prechilled broiler carcasses were 2.7 ± 0.4, 2.7 ± 0.4 and 2.3 ± 0.2 log_{10} cfu/part, respectively.

Raj (2002) estimated the *E. coli* count of 60 chicken carcasses from three sources and reported the overall mean count as 2.20 ± 0.27 log_{10} cfu/ml.

Fluckey *et al.* (2003) determined the level of generic *E. coli* from broiler carcasses sampled before and after evisceration and reported that the former samples had an *E. coli* count of 3.74 log_{10} cfu/ml of rinse and the count on the latter samples was 3.08 log_{10} cfu/ml.

2.1.4 Faecal Streptococcal Count

Panda (1971) reported that dressed chicken collected from three shops in Bangalore had average *faecal streptococcal count* of 1.2×10^3, 8.2×10^4 and $1.9 \times 10^3/cm^2$, respectively. The count of the samples from two shops in Mysore was 1.7×10^4 and $2.7 \times 10^3/cm^2$, respectively.

Cox *et al.* (1983) estimated the Karl Friedrich (KF) streptococcal count of the various raw ingredients used to make chicken patty products and reported that the count on broiler thigh meat varied from 3.5 to 3.6 log_{10}/g.

Anand *et al.* (1990) reported that the mean KF streptococcal count of dressed broiler chicken meat used in the preparation of chicken sausages was 2.3 log/g.

Anand *et al.* (1991) evaluated the KF streptococcal count of manually deboned meat from culled birds and reported that the samples had a mean count of 2.09 ± 0.04 log/g.

Anand *et al.* (1992) determined the KF streptococcal count of five dressed birds and reported the mean count as 2.97 ± 0.07 log_{10} cfu/g.

Murugkar *et al.* (1993) evaluated the microbiological profile of various poultry products manufactured under Indian conditions. The study revealed that out of 18 whole dressed chicken carcasses examined, two were positive for faecal streptococci and the mean count of the organism was 4.29 log_{10} cfu/g.

Raj (2002) reported the overall mean faecal streptococcal count of 60 chicken carcasses from three sources as 4.32 ± 0.09 log_{10} cfu/ml.

2.2 ISOLATION OF BACTERIA

2.2.1 *Escherichia coli*

Panda (1971) isolated various microorganisms from the skin surface of dressed chicken collected from shops supplying birds immediately after dressing and reported that four per cent of the isolates were *Escherichia*

Bok *et al.* (1986) studied the incidence of various food-borne pathogens on 102 retail broiler samples and reported that 44 (6.85 per cent) out of 642 isolates were *E. coli.*

Doyle and Schoeni (1987) investigated the presence of *E. coli* 0157: H7 in retail fresh meats and poultry. The study revealed that four (1.5 per cent) of 263 poultry samples had *E. coli* 0157: H7

Nair *et al.* (1990) reported the isolation of 150 *E. coli* strains from dressed chicken carcasses obtained from the local market and the poultry processing unit at CFTRI, Mysore. The serotypes *E. coli* 078, *E. coli* 0154 and *E. coli* 0156 from the former source and *E. coli* 026 and *E. coli* 028 from the latter source were enteropathogenic.

Turtura (1991) isolated 369 strains of bacteria from slaughtered poultry and reported that 65 were identified as *E. coli.*

Murugkar *et al.* (1993) reported the isolation of *Escherichia coli* from three out of 18 whole dressed chicken samples.

Samadpour *et al.* (1994) studied the presence of Shiga like toxin producing *Escherichia coli* in retail fresh sea food, beef, lamb, pork and poultry and observed that four (12 per cent) of 33 chicken samples were positive.

Vorster *et al.* (1994) reported that *Escherichia coli* was isolated from 34 (79.1 per cent) out of 43 broiler samples.

Sharma *et al.* (1995) investigated the incidence of *Escherichia coli* in milk, meat and meat products. The study revealed that 14 (60.87 per cent) out of 23 chicken meat samples were positive for *E. coli* and the isolates belonged to serotypes 05, 062, 0158 and 0162.

Geornaras *et al.* (1996) assessed the bacterial population associated with poultry processing in a South African abattoir. They found that 31.0 per cent of the bacteria isolated using TSAYE (tryptone soya agar supplemented with yeast extract) and 80.0 per cent of the isolates obtained using VRBGA (violet red bile glucose agar) media were *E. coli.*

Pattnaik *et al.* (1997) evaluated the microbial load on chicken carcasses processed in slaughter units of Bhubaneswar city. They reported that four per cent of the isolates were *E. coli.*

Hang'ombe *et al.* (1999) assessed the bacterial contamination of poultry meat packaged at a Zambian poultry abattoir. During the investigation they isolated 521 bacterial strains, of which 217 (41.7 per cent) were *E. coli.* Of the isolates 30 were serotyped against specific antisera, of which eight were serotyped and 22 were untypable. The serotypes identified included O111:K58, O26:K60 and O26: K.

Banerjee *et al.* (2001) studied meat of different animals and its products to assess the occurrence of Vero toxin producing *E. coli.* The organism was isolated from 12 (44.44 per cent) out of 27 chicken meat samples and one of the isolates was verotoxigenic *E. coli* 086.

Soriano *et al.* (2001a) determined the incidence of microbial flora in lettuce, meat and Spanish potato omelette and reported the isolation of *E. coli* from one (2.5 per cent) of 40 chicken samples.

Yashoda *et al.* (2001) assessed the microbiological quality of broiler chicken carcasses processed hygienically in a small-scale poultry processing unit and reported that *E. coli* was absent in all samples.

Raj (2002) isolated 53 *E. coli* from 60 chicken carcasses. Only 44 isolates were serotyped and they belonged to O5, O8, O14, O25, O33, O41, O49, O66, O78, O81, O84, O85, O91, O116, O121, O131, O132, O146, O150, O157 and O161. Six isolates were identified as rough and two were untypable.

2.2.2 *Salmonella*

Jayaraman *et al.* (1972) examined 519 samples of intestinal contents, liver, heart blood, urine and poultry meat. They reported the isolation of *S. newport* from one of the four poultry meat samples.

Al-Hindawi and Rished (1979) investigated the occurrence and distribution of *Salmonella* spp. in 353 local food samples from Baghdad city. The study revealed that *Salmonella* was present in six (12.8 per cent) of 47 raw chicken meat samples.

Al-Rajab and Hussain (1982) studied the occurrence of *Salmonella* serotypes in 600 local food samples collected from commercial sources in the city of Baghdad. They reported the isolation of *Salmonella* from nine (18 per cent) of 50 chicken skin and 10 (20 per cent) of 50 chicken wings.

Campbell *et al.* (1983) surveyed the levels of coliforms, *Escherichia coli* and *Salmonella* spp. on eviscerated chicken from selected chicken eviscerating plants in 1979 and the data from this survey was compared with that conducted in 1969. The study revealed that the carcasses at the entry of the chillers, after spray washing had a *Salmonella* incidence of 20.5 per cent and 5.5 per cent in 1969 and 1979 respectively.

Bok *et al.* (1986) analysed the incidence of food borne pathogens on 102 retail broilers and observed that 49 per cent of the samples were contaminated

with *Salmonella* and isolated 136 *Salmonella* which were belonging to 16 serotypes.

Yagoub and Mohamed (1987) isolated *Salmonella* from three (4.54 per cent) out of 66 dressed carcass skin and three (3.75 per cent) out of 80 carcass drip water samples. The isolates belonged to serotypes *S. emek*, *S. heidelberg* and *S.*16: L, V: *monophas*

Boer and Hahne (1990) studied cross contamination of raw chicken products with *Campylobacter jejuni* and *Salmonella* spp. during food preparation. They isolated *Salmonella* from 44 (54 per cent) out of 81 chicken products, consisting of drumsticks, wings, livers and fillets from retail outlets.

Lillard (1990) determined the incidence of *Salmonella* on broiler carcasses at various stages of processing and reported that 10 (11.9 per cent) of 84 post pick carcasses and 12 (14.3 per cent) of 84 pre-chilled carcasses were positive for the organism.

Nair *et al.* (1990) examined 50 samples each of dressed chicken carcasses produced from local market and the poultry-processing unit at CFTRI, Mysore. *Salmonella* was isolated from two of the carcasses collected from the former source and belonged to serotypes *S. virchow* and *S. newport*, but none of the samples from the latter source had *Salmonella*.

Izat *et al.* (1991) evaluated broiler carcasses at retail for the incidence, number and serotypes of *Salmonella*. The study revealed that five of 24, six of 24 and 10 of 24 frozen chicken broilers respectively from brands A, B and C were positive for *Salmonella*. The serotypes isolated were *S. typhimurium*, *S. paratyphi* and *S. arizonae*.

James *et al.* (1992) determined the prevalence of *Salmonella* on raw poultry carcasses at different stages of processing. They reported the isolation of

the organism from 58 per cent of pre eviscerated and 48 per cent of pre-chilled carcasses.

Jetton *et al.* (1992) assessed the effect of rinse media and enumeration method on the recovery of salmonellae from 30 chilled broiler carcasses. The organism was detected on six carcass halves rinsed with physiological saline (PS) and on eight carcass halves rinsed with distilled water (DW), by MPN method. However, when centrifugation plating onto dulcitol novobiocin agar was used, salmonellae were found on 10 carcass halves rinsed with PS and none rinsed with DW.

Paturkar *et al.* (1992) studied the occurrence of *Salmonella* in 96 meat samples from different parts of Bombay and reported the isolation of *S. butantan* from one of the chicken samples.

Whittemore (1993) detected *Salmonella* in 22 per cent of 24 broiler carcass rinse samples by single dilution series most probable number technique.

Jerngklinchan *et al.* (1994) examined 1135 samples of raw chicken meat, giblets and cooked chicken products for the presence of salmonellae. The organism was isolated from 467 (66 per cent) out of 705 chicken meat samples collected from various sources such as open market, super market and processing plant. The serotypes isolated in the order of occurrence were *S.blockley, S. virchow, S. enteritidis, S. agona, S. anatum, S. hardar, S. kentucky, S. paratyphi B, S. derby, S. amsterdam, S. montevideo, S. emek* and *S. stanley.*

Plummer *et al.* (1995) evaluated the level of *Salmonella* contamination in retail chicken products. They found that five out of 19 fresh whole birds and 10 out of 31 frozen whole birds collected from super market and four out of 14 whole birds obtained from butcher's shop had *Salmonella*.

Sharma *et al.* (1995) reported the isolation of *Salmonella* from 8 (10.52 per cent) out of 76 frozen chicken carcasses. The isolates were serotyped as *S. typhimurium, S. saintpaul, S. indiana* and *S. stanley.*

Cason *et al.* (1997) determined the incidence of *Salmonella* on broiler carcasses taken from the processing lines after defeathering (post pick) before chilling (pre chill) and after chilling (post chill). They reported that seven (23 per cent) out of 30 post pick carcasses, 18 (20 per cent) out of 90 pre chill carcasses and 17 (19 per cent) out of 90 post chill carcasses had salmonellae.

Zivkovic *et al.* (1997) analysed 1057 samples of chicken meat and products for the presence of salmonella serovars. The study revealed that three (11.54 per cent) out 26 dressed chicken meat and 26 (3.87 per cent) out of 672 fresh carcasses were positive for salmonellae and the serovars were *S. typhimurium, S. hadar* and *S. virchow.*

Sarlin *et al.* (1998) evaluated the whole carcass rinse procedure versus excised skin or swabs from the thoracic inlet region for the sensitivity of *Salmonella* detection and also evaluated the effect of crop removal or chill tank exposure on the frequency of *Salmonella* recovery from commercially processed broiler carcasses. *Salmonella* were isolated from nine (32.14 per cent) of 28 cutaneous swab samples of pre-chilled carcasses. The organism was isolated from five (17.86 per cent) of 28 cut surface swab samples collected from pre-chilled carcasses and an equal number of organisms was isolated from 28 thoracic swab samples of pre chilled carcasses. The per cent of samples which revealed the organism in excised skin and carcass rinse samples collected from pre-chilled carcasses were 70, 89 and 29, respectively. The organism was isolated from 17 (68 per cent) out of 25 post-chilled carcass rinse.

Telo *et al.* (1998) investigated 80 imported poultry meat samples and *Salmonella* was detected in 10 (12.5 per cent) samples. The most frequent strain encountered was S. *enteritidis.*

Duffy *et al.* (1999) tested 180 samples of retail meat and poultry for the presence of naturally occurring *Salmonella* spp. The incidence of organism in chicken meat samples was 26.4 per cent. The serotypes identified include *S. bredeney, S. kentucky, S. enteritidis, S. london* and *S. schwartzangram.*

Hang'Ombe *et al.* (1999) reported that out of 521 isolates recovered from 382 poultry carcasses, 107 (20.53 per cent) were *Salmonella* spp., belonging to serotypes S. *mbandaka, S. enteritidis, S. infantis* and *S. gallinarum.*

Peresi *et al.* (1999) examined chicken carcasses produced in the Sao Jose do Rio Preto area, Brazil and reported the isolation of *Salmonella* from 87 (54.38 per cent) out of 160 carcasses. Eighteen serovars were identified and the most frequently isolated serovar was S. *enteritidis.*

Uyttendaele *et al.* (1999) examined 772 samples of poultry carcasses and products from the retail market for the presence of *Salmonella.* Out of 133 chicken carcasses six (4.5 per cent) were positive for *S. enteritidis* while other *Salmonella* spp. were detected in 39 (29.3 per cent) samples.

Chang (2000) reported the incidence of *Salmonella* spp. in 25.9 per cent of retail broiler chicken samples. The seven isolates obtained in the study belonged to serotypes *S. enteritidis* (5), *S. virginia* (1) and *S. virchow* (1)

Konwar and Joshi (2000) compared the effectiveness of enrichment broths on the recovery of *Salmonella* from naturally contaminated raw meat. The organism was detected in one of the 38 chicken samples using Rappaport – Vassiliadis medium at 42°C (RV_{42} medium).

Harrison *et al.* (2001) determined *Salmonella* spp. contamination associated with 300 raw chicken obtained from two sources. The organism was present in 33 per cent of 175 chicken samples from super markets and 24 per cent of 125 samples from butcher's shops.

Mikolajczyk and Radkowski (2002) analysed chicken carcasses at various stages of processing and reported that *Salmonella* spp. were found in six per cent of the cloacal samples collected after stunning the chicken. The per cent of chicken carcass swab samples taken from the skin surface and interior of the body after evisceration, skin surface and interior of the body cavity before cooling and skin surface and interior of the cavity after cooling which revealed the presence of salmonellae were 24, 52 and 13, respectively.

Simmon's *et al.* (2003) studied the recovery of *Salmonella* from retail broilers by a whole carcass enrichment procedure and reported that 85 (33.9 per cent) of 251 carcasses were positive for salmonellae.

2.2.3 *Staphylococcus aureus*

Panda (1971) determined the incidence and distribution of various types of microorganisms associated with the dressed chicken sold through retail outlet and reported that 12 per cent of the isolates were pathogenic staphylocococci.

Pandurangarao (1977) examined 66 buffalo meat, 55 pork, 12 mutton and seven chicken meat samples collected from the retail market in Bareilly for enterotoxigenic staphylococci and reported that one of the chicken meat samples had coagulase positive staphylococci.

Nkanga and Uraih (1981) carried out an investigation to detect and enumerate *Staphylococcus aureus* from meat samples sold in the traditional markets in Benin city, Nigeria. The organism was isolated from 54.1 per cent of 24 frozen chicken samples.

Bok *et al.* (1986) reported the isolation of 31 staphylococci from 102 broiler samples and concluded that 19 per cent of these isolates were coagulase positive.

Murugkar *et al.* (1993) isolated *Staphylococcus aureus* from four out of 18 whole dressed chicken samples.

Vorster *et al.* (1994) reported the occurrence of *Staphylococcus aureus* in 17 (39.5 per cent) of the 43 broiler samples.

Pattnaik *et al.* (1997) isolated various bacteria from the leg meat of hot (unchilled) eviscerated carcasses of one-month-old broiler chicken and observed that, of the 50 isolates recovered, 14 (28 per cent) were *Staphylococcus aureus.*

Hang'Ombe *et al.* (1999) identified the presence of 13 bacterial species on chicken carcasses obtained from the market in Lusaka, Zambia. Out of 521 isolates recovered from 382 carcasses, 13 were *Staphylococcus* spp. and eight of these were coagulase positive.

Capita *et al.* (2001) evaluated the microbiological quality of retail poultry carcasses in Spain. They concluded that all the 40 eviscerated and refrigerated broiler chicken carcasses obtained from five retail outlets had *Staphylococcus aureus.*

Soriano *et al.* (2001a) reported the isolation of *Staphylococcus aureus* from one (2.5 per cent) of 40 raw chicken samples.

2.2.4 *Listeria monocytogenes*

Pini and Gilbert (1988) examined 100 raw chicken. *Listeria monocytogenes* was isolated from 33 (66 per cent) of 50 fresh chicken and 27 (54 per cent) of 50 frozen samples. Other *Listeria* spp. including *L. welshimeri, L. seeligeri* and *L. innocua* were isolated from 28 per cent of the samples.

Bailey *et al.* (1989) studied the recovery and serotype distribution of *L. monocytogenes* from 90 broiler chicken carcasses obtained from South Eastern United States. They isolated the organism from 23 per cent of the carcasses and the isolates were serotyped as 1/2 b (64 per cent) and 1/2 c (18 per cent) .

Genigeorgis *et al.* (1989) investigated 160 packages of poultry legs (drumsticks), wings and whole birds purchased from three supermarkets for the

presence of *Listeria* spp. and reported the isolation of the organism from 40.6 per cent of the samples. *L monocytogenes, L. innocua* and *L. welshimeri* were isolated from 13.1, 26.3 and 1.3 per cent of the chicken parts.

Genigeorgis *et al.* (1990) analysed 180 packages of raw turkey products and *Listeria* spp. were isolated from 27 (45 per cent) of 60 samples of wings, 17 (28.3 per cent) of 60 drumsticks and 14 (23.3 per cent) of 60 tails. *L. monocytogenes* was detected in 20, 13.3 and 11.7 per cent of wings, drumsticks and tails, respectively. *L. innocua* was detected in five per cent of the wings whereas; L. *welshimeri* was isolated from 20 per cent of the wings, 15 per cent of drumsticks and 11.7 per cent of tails.

Seneviratna *et al.* (1990) determined the prevalence of *Listeria* spp. in foods of animal origin and reported the isolation of *L. monocytogenes* from one (5.9 per cent) and *L. innocua* from three (17.7 per cent) of 17 avian meat samples.

Varabioff (1990) investigated 80 frozen and 48 fresh chicken carcasses and reported the isolation of *L. monocytogenes* from 12 (15 per cent) of the former samples. *L. innocua* was also isolated from 14 (17.5 per cent) of three samples. One (2.1 per cent) of the fresh chicken yielded *L. monocytogenes* and five (10.4 per cent) had *L.innocua*.

Wong *et al.* (1990) examined a variety of foods for the incidence of *L. monocytogenes* and found that the organism was present in 50 per cent of 16 chicken carcasses.

Kwiatek *et al.* (1992) examined 593 samples of raw meat, poultry and 97 raw milk samples and reported the isolation of *L. monocytogenes* from 60 per cent of 60 poultry meat samples.

Wang *et al.* (1992) tested 21 chicken carcasses and reported the isolation of *L. monocytogenes* from one (4.7 per cent) of the samples and *Listeria* spp. from 11 (52.4 per cent) samples.

Ojeniyi *et al.* (1996) examined 320 samples of ready to cook poultry collected from eight abattoirs and reported the isolation of *L. monocytogenes* from 29 (9.1 per cent) samples.

Johansson (1998) compared Palcam medium (PAL), Oxford medium (OX), *Listeria* selective medium based on lithium chloride and ceftazidime (LA) and *L. monocytogenes* blood medium (LMBA) for the isolation and enumeration of *L. monocytogenes* from foodstuffs. Out of 142 fresh broiler cuts examined, 73 were positive for *L monocytogenes* and the number of samples from which the organism was isolated by using OX, LA and LMBA were 49, 57 and 73, respectively.

Uyttendaele *et al.* (1999) reported the isolation of *L. monocytogenes* from 70 out of 133 chicken carcasses collected from the retail market.

Choi *et al.* (2001) examined a variety of foods and reported the isolation of *Listeria* spp. from 10 (33.3 per cent) of 30 chicken samples. The isolates consisted of *L. monocytogenes, L. seeligeri, L. innocua* and *L. welshimeri*.

Miettinen *et al.* (2001) examined 61 raw broiler cuts collected from retail stores to determine the level of contamination of the samples with *L. monocytogenes*. Of these samples, 38 (62 per cent) had *L. monocytogenes*. The per cent isolation of the organism from leg, drumstick, breast and wings was 68, 67, 52 and 50, respectively.

Soriano *et al.* (2001b) studied the incidence of *Listeria* in 103 raw and ready to eat foods from restaurants. They reported that one of the five raw chicken had *L. grayi* and none of the samples yielded *L. monocytogenes*.

Antunes *et al.* (2002) analysed 63 Portuguese poultry carcasses and reported that cent per cent samples had *Listeria* spp. and *L. monocytogenes* was detected in 43 per cent of the samples. Other species of *Listeria* isolated from the samples included *L. innocua*, *L. welshimeri* and *L. seeligeri*.

2.3 BACTERIAL COUNT FROM THE ENVIRONMENTAL SAMPLES AND PROCESSING EQUIPMENT

Anad *et al.* (1989) analysed the bacterial quality of scald and wash water samples and reported that the mean total plate count of the former samples before beginning the scalding was 2.6 $\log_{10}$/ml, while the count increased to 5.6 $\log_{10}$/ml after scalding 100 birds. The average coliforms count of the sample before beginning the operation and after scalding 100 birds was zero and 3.7 $\log_{10}$/ml, respectively. The staphylococcal count of the samples before scalding the birds was 2.0 $\log_{10}$ cfu/ml and was increased to 3.9 $\log_{10}$ cfu/ml after scalding 100 birds. The mean total viable count, coliforms count and staphylococcal count in the wash water before washing the carcass was two, zero and zero $\log_{10}$ cfu/ml, respectively and was increased to 4.9, 2.9 and 3.6 $\log_{10}$ cfu/ml respectively, after washing 100 carcasses.

Lillard (1990) examined the bacterial load of the immersion scalder and immersion chiller. The study revealed that the former samples had a mean aerobic bacterial count and enterobacteriaceae count of 5.42 ± 0.53 and 2.71 ± 0.58 $\log_{10}$ cfu/ml, respectively. The corresponding counts of the samples from the latter source were 3.57 ± 0.36 and 2.84 ± 0.47 $\log_{10}$ cfu/ml, respectively. *Salmonella* was detected in 15.3 per cent of the immersion scald water and in 52.8 per cent of chill water samples.

Rao and Ramesh (1992) analysed water, knives, floor, wall, processing equipments and hands of workers before and after slaughtering operations to identify the critical control points of microbial contamination in the slaughter line. The study indicated that the total viable count of the samples before

slaughtering operations was at the level of 10^2 cfu/cm^2 or per ml in the floor washings, wall and equipments. The mean count in water and knives samples was at the level of 10^1 cfu/cm^2 or per ml. They observed a four fold increase in the count of the samples collected after slaughter.

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

Abu-Ruwaida *et al.* (1994) evaluated the microbial load of water and the swab samples from the equipment and utensils in different locations in the processing plant on two days. The mean aerobic bacterial count of water samples from main tank, spray water, dripping water and scalding water on day one ranged from 2.2 to 8.0 $\log_{10}$ cfu/ml and the count on the second day varied between 2.1 and 7.6 $\log_{10}$ cfu/ml. Coliforms, *E. coli, Staphylococcus aureus, Campylobacter* and *Salmonella* were absent in main tank water and spray water but they were detected in dripping water and scalding water samples. The mean aerobic bacterial count of air sampled at different points of the processing line ranged from six to 300 and one to 300 cfu/min on days one and two, respectively. The air samples collected from the slaughtering, scalding, defeathering and evisceration areas were contaminated with different types of airborne microflora. The overall contamination level of aerobic bacteria on equipments and utensils in the evisceration area ranged from 4.1 to 7.7 $\log_{10}$ cfu/cm^2 on day one and the corresponding count in the above area on day two was varied between 4.0 to 7.4 $\log_{10}$ cfu/cm^2.

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

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

Gill *et al.* (1999) analysed the samples collected from cleaned processing equipment and steel mesh gloves of workers in a sheep-carcass breaking process. They reported that the samples from conveyor belt support bones had a mean total aerobic count of two to five $\log_{10}$ cfu/cm^2 and the count on the steel mesh gloves was seven to nine $\log_{10}$ cfu/cm^2. No bacteria were recovered from samples of counter weighed saw and cutting board. Coliforms and *E. coli* counts on the steel mesh gloves were greater than 3.0 $\log_{10}$ cfu/cm^2.

2.4 STANDARDS FOR BACTERIAL COUNT

The level and type of bacterial contamination on carcasses have a significant role on the shelf life of meat and its products. A very few organizations or scientists have developed microbiological standards or limits or criteria for meat and its products.

2.4.1 Total Viable Count

Carl (1975) suggested the aerobic count limit of fresh meat as five million per gram. The microbiological standard prescribed by Government of India (1991) states that the total viable count in three out of five raw meat samples

should not exceed 10^6 per gram and the remaining two samples can have the count upto 10^7 per gram (Rao *et al.*, 1998). Gracey *et al.* (1999) reported the recommendation of ICMSF (1986) as the frozen poultry when examined by rinsing should give a count at 20°C of less than 10^7/ml of the rinsing solution.

2.4.2 *Escherichia coli* Count

Carl (1975) suggested the *Escherichia coli* limit for fresh meat as 50 per gram. The Government of India (1991) has prescribed the microbiological standards for fresh and frozen meat which states that the *Escherichia coli* count in three out of five samples shall not exceed 10 per gram and in the remaining two samples the count can be upto 100 per gram (Rao *et al.*, 1998).

2.4.3 *Salmonella*

According to the microbiological standards prescribed by Government of India (1991), *Salmonella* should be absent in all the five samples of raw meats tested (Rao *et al.*, 1998). Gracey *et al.* (1999) recorded the recommendation of ICMSF (1986) which states that *Salmonella* should be detected in not more than one of five 25 g samples of poultry meat. According to the Food Act, Government of Mauritius (1998) *Salmonella* should be absent in 25 g of raw meat and poultry.

Materials and Methods

3. MATERIALS AND METHDOS

The shelf life of chicken carcasses and its products and also the consumer safety due to the consumption of these products primarily depend on their microbial quality. The microbial quality of the carcasses depends on the level of contamination from the feathers, defeathering machine, hygienic practices of personnel engaged in the slaughter and dressing of chicken and also the environment. Considering the above facts bacterial quality of chicken carcasses produced in a meat processing plant located at Cochin was evaluated at two points on the production line.

In the plant live birds of about six to eight weeks of age are brought from farmers as well as plant's farm and processed at the rate of 500 to 1000 birds per day. The various steps followed in the production of dressed chicken are sticking, bleeding for one to two minutes, scalding at 62 to 65°C for one to two minutes, defeathering, washing, removal of head and feet, immersion in chilled water, evisceration, washing, packing and air chilling at 0°C. The scalding tank water is changed daily after the morning and afternoon sections of scalding.

3.1 COLLECTION OF SAMPLES

During the study a total of 60 dressed chicken carcasses consisting of 30 each were randomly collected after the removal of head and feet (ARHF) and after evisceration (AE), to evaluate the bacterial quality. On the day of sampling only three samples were collected from each step and the experiment was repeated 10 times.

In order to collect the samples, the selected carcass after the removal of head and feet was transferred into a sterile polyethylene bag and added 200 ml of 0.1 per cent sterile peptone water diluent (James *et al.*, 1992) and was thoroughly agitated by holding between the hands for a period of one minute. The rinsate

was then transferred into a sterile conical flask and brought to the laboratory in a thermocool container.

In the case of carcasses collected after evisceration, they were also placed in sterile polyethylene bags and the diluent was poured into the bag in such a way that sufficient quantity of diluent should reach the body cavity of the carcass and agitated thoroughly as described earlier. The rinsate was transferred and transported to the laboratory as described before.

3.2 PROCESSING OF SAMPLES

In order to evaluate the various bacterial counts, 25 ml of the rinsate collected from each carcass was transferred into 225 ml of 0.1 per cent sterile peptone water so as to form one in 10 dilution. From this, transferring one ml of the inoculum into nine ml of the diluent made further serial 10 fold dilutions. The serial dilution of each sample was made up to 10^7 (Swanson *et al.*, 2001). The selected serial dilutions of each sample were used to enumerate the levels of various bacteria per ml of carcass rinse.

3.3 BACTERIAL COUNT

The whole carcass rinse prepared from each carcass was used to estimate the total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC).

3.3.1 Total Viable Count

Pour plate technique described by Swanson *et al.* (2001) was used to estimate the total viable count of each sample. From the selected 10 fold dilutions of each sample, one ml 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 petri dishes in clockwise, anticlockwise, forward and backward manner. The

inoculated plates were allowed to solidity at room temperature and were then incubated at 37°C for 24 h. At the end of incubation period, petri dishes with a colony count between 30 and 300 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 expressed as log_{10}cfu/ml.

3.3.2 Coliforms Count

Coliforms count (CC) was estimated as per the procedure described by Nordic Committee on food analysis (1973). From the selected dilution, 0.1 ml of the inoculum was transferred onto duplicate plates of violet red bile agar (VRBA) (Hi-media) 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 at least 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 expressed as log_{10}cfu/ml.

3.3.3 *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 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 centre and a distinct indelible ink, greenish black metallic sheen on deflected light were counted as *E. coli*. The number of organisms per ml of sample was estimated as described for CC and expressed as log_{10}cfu/ml.

3.3.4 Faecal Streptococcal Count

Faecal streptococcal count (FSC) of the sample was estimated by the method described by Nordic committee on food analysis (1968b). To estimate the number of organisms in the carcass rinse, 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 on to 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 three 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.

3.4 ISOLATION AND IDENTIFICATION OF BACTERIA

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

3.4.1 *Escherichia coli*

To isolate *E. coli*, a loopful of the carcass rinse was inoculated onto 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 characteristics of *E. coli* was transferred onto nutrient agar slants and incubated at 37°C over night. 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 subjected to Eijkman test (Barrow and Feltham, 1993). The isolates identified as *E. coli* were serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh.

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

	Characteristics/Reactions
Dressed chicken carcass	
↓	
Whole carcass rinse with 200 ml sterile 0.1 per cent peptone water	
↓	
Inoculate a loopful of the rinse on EMB agar	
↓	
Select suspected colonies	Colonies with dark centre 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	+
Glucose	+
Mannitol	+
Maltose	+
Inositol	-

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

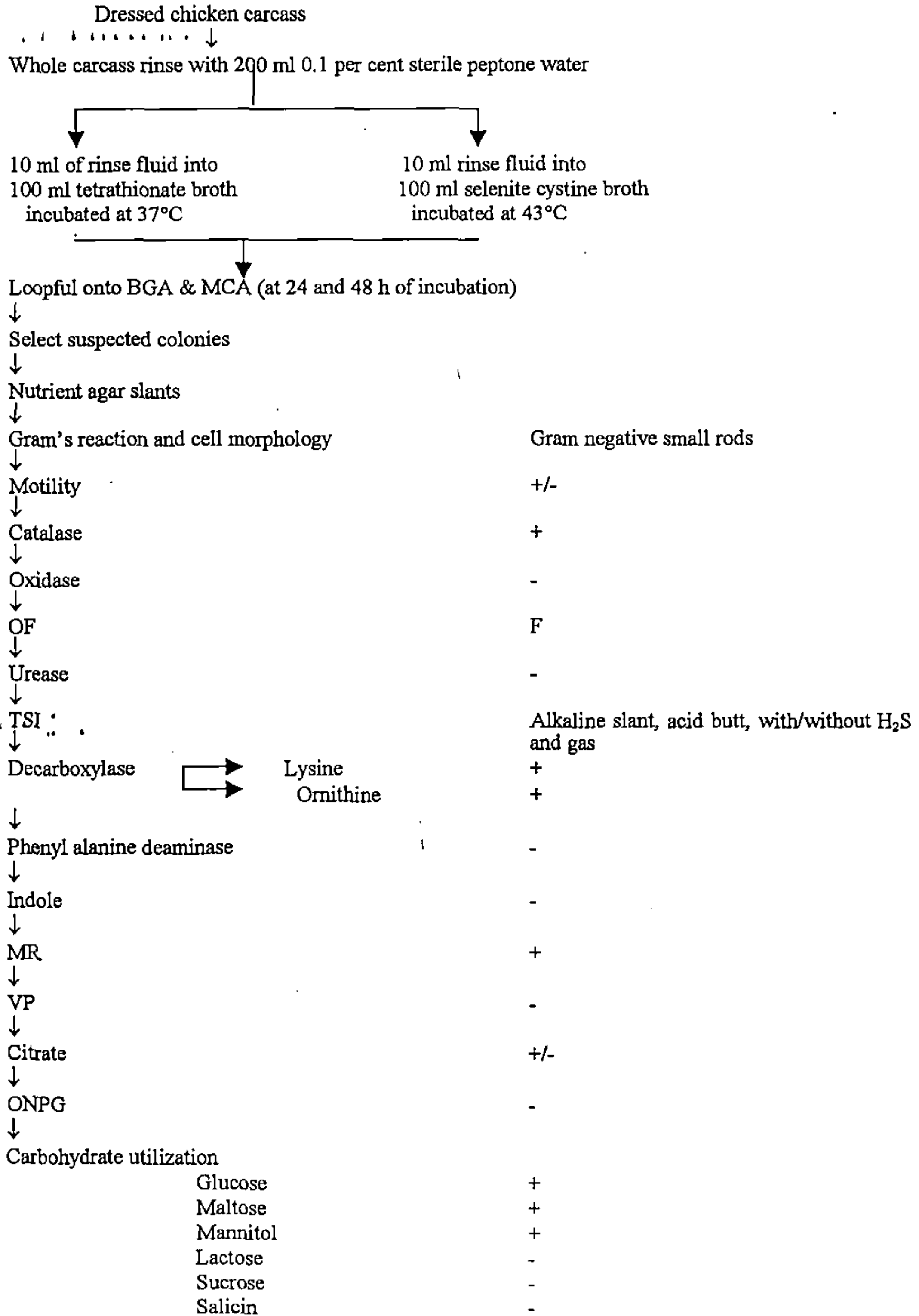
3.4.2 Salmonellae

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 broth was incubated at 37°C for 48 h and the flask containing selenite cystine broth was incubated in a water bath at 43°C for 48 h. At the end of 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 Mac Conkey agar (MCA) (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 one to two mm, surrounded by a pink or red hue on BGA and transparent colourless colonies with opaque center on MCA were selected (Andrews et al.,2001). The selected colonies were transferred to nutrient agar slants and incubated at 37°C overnight and stored at refrigeration temperature for further characterization of the isolates. The cultural, morphological and biochemical characteristics of the isolates were identified according to the procedure described by Edwards and Ewing (1972) and Barrow and Feltham (1993) and are shown in flow chart 2. The isolated salmonellae were serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh.

3.4.3 *Staphylococcus aureus*

For the isolation of *Staphylococcus aureus,* a loopful of the carcass rinse was inoculated on to Baird-Parker (BP) agar medium (Hi- media) and incubated at 37°C for 48 h (Lancette and Bennett, 2001). At the end of incubation, colonies showing characteristic appearance (circular, smooth, convex, moist, two to three mm in diameter on uncrowded plates, gray black to jet black, frequently with light coloured margin, surrounded by opaque zone and frequently with outer clean zone or halo) on BP agar medium were selected and transferred to nutrient

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



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

agar slants and incubated at 37°C overnight. The isolates were stored at refrigeration temperature. Characterization and identification of the isolates were done following the procedure described by Barrow and Feltham (1993) and are shown in the flow chart 3. The isolates were identified based on the cultural, morphological and biochemical characteristics.

3.4.4 *Listeria monocytogenes*

The procedure used for the isolation and identification of *L. monocytogenes* was similar to that described by Wang et al (1992). From each whole carcass rinse, 10 ml was transferred into a sterile conical flask containing 90 ml of Listeria enrichment broth (LEB) (Hi- media) and mixed thoroughly and incubated at 30°C for seven days. After second and seventh days of enrichment samples were streaked onto Oxford and polymyxin B acriflavin lithium chloride ceftazidime aesculin mannitol (PALCAM) agar (Hi- media) plates. The plates were incubated at 30°C for 24-48 h. At the end of incubation, those colonies with a black zone on Oxford agar and those with cherry red background on PALCAM agar were inoculated onto lithium phenyl ethanol chloride moxalactam (LPM) agar (Hi- media). The plates were incubated at 30°C for 24 h and examined under Henry's oblique lighting technique. Typical grayish blue colonies were picked and streaked onto trypticase soy agar (TSA) slants and incubated at 30°C for 24 h and stored at refrigeration temperature for further characterization of the isolates. The isolates were subjected to various tests as shown in the flow chart 4.

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

	Characteristics/Reactions
Dressed chicken carcass	
↓	
Whole carcass rinse with 200 ml sterile 0.1 per cent peptone water	
↓	
A loopful onto BP agar medium	
↓	
Select suspected colonies	Circular, smooth, convex, moist, 2-3 mm in diameter, grey black to jet black with outer clear zone
↓	
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

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

Characteristics/Reactions

Dressed chicken carcass
↓
Whole chicken rinse with 200 ml of sterile 0.1 per cent peptone water
↓
10 ml of rinse fluid to 90 ml Listeria enrichment broth

↓	↓
Loopful onto Oxford medium on second and seventh day	Loopful onto PALCAM medium on second and seventh day

↓
LPM Medium
↓
Henry's oblique lighting technique
↓
Selected Greyish blue colonies
↓
Trypticase Soy Agar
↓

Test	Characteristics/Reactions
Gram's reaction and cell morphology	Gram positive short rods
↓ Catalase	+
↓ Oxidase	-
↓ OF	F
↓ Motility	Motile at 20-25°C
↓ MR	+
↓ VP	+
↓ Citrate	-
↓ Urease	-
↓ Phosphatase	+
↓ Aesculin hydrolysis	+
↓ Hippurate hydrolysis	+
↓ Carbohydrate utilization	
Mannitol	-
Rhamnose	+
Xylose	-

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

3.5 CHARACTERIZATION AND IDENTIFICATION OF ISOLATES

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

3.5.1 Primary Identification Tests

1. Catalase test

a) 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 indicated a positive reaction (Barrow and Feltham, 1993).

b) 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 was indicated by the development of effervescence immediately .

2. Gram staining

The procedure used for gram staining was as follows:

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

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

c. Poured off the stain and washed with water.

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

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

f. Washed the smear and counter stained with dilute carbol fuchsin 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 up to a depth of five mm. Motility was indicated by a spreading growth into the medium from the line of inoculation and growth of non-motile organisms was confined to the stab

4. Oxidase test

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

5. Oxidation – Fermentation test

Each isolate was inoculated into duplicate tubes of Hugh and Leifson'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 three 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 was taken as oxidation whereas a change

in colour from green to yellow in both the tubes was regarded as fermentation. Absence of colour change in both tubes indicated no action on carbohydrates.

3.5.2 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 indicated 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, 0.25 ml of Nessler's reagent was added. Arginine hydrolysis was 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 per cent 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 indicated acid production and the production of gas was indicated by the appearance of air bubbles in the inverted Durham's tube. Anaerobic condition of the medium was provided by adding a layer of sterile molten soft paraffin to a depth of about one centimeter above the media.

4.Citrate utilization test

A light suspension of the organism was made in normal saline and was inoculated with a straight wire onto the slope of Simmon's citrate agar. The inoculated medium was incubated at 37°C and examined daily up to seven days.

The ability of the organism to utilize citrate as the sole source of carbon was 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

a) Slide test

A small quantity of the culture was emulsified in a drop of saline on a microscope slide to produce a thick suspension. The suspension was stirred with a straight wire dipped in rabbit plasma. A positive result was indicated by macroscopic clumbing within five seconds and delayed clumbing was considered as a negative reaction.

b) Tube test

Mixed 0.5 ml undiluted rabbit plasma with an equal volume of an 18 to 24h 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 contained lysine and other contained ornithine. The third tube was taken as the control. The organism was inoculated through the paraffin layer and incubated at 37°C for five days. In a positive reaction, the medium first turned yellow and then became purple and the control tubes remained yellow.

7. Eijkman test

Each test organism was inoculated into tubes containing Mac Conkey broth with inverted Durham's tube, warmed to 37°C and incubated at 44 ± 0.1°C

in a water bath for 48 h. Production of both acid and gas indicated a positive reaction.

8. Gelatin hydrolysis/liquefaction

Each isolate was inoculated into nutrient gelatin and incubated at 37°C for 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 was 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 was indicated by growth and the development of a pink colour due to alkali production.

10. Indole production

The isolate was inoculated into nutrient broth 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 indicated 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 indicated 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 was indicated by the development of an yellow colour due to the production of O-nitrophenol.

13. Phenylalanine deamination

The phenylalanine agar slope was heavily inoculated with the test organism and incubated at 37°C for overnight. At the end of incubation, 0.2 ml of 10 per cent aqueous solution of ferric chloride was poured over the slope. A positive result was 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.

16. Urease activity

Slope of Christensen's urea agar was heavily inoculated with the test organism and incubated at 37°C. The tubes were examined after 4 h of incubation and daily for five days. Development of a red colour in the medium indicated 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 five per cent α naphthol solution and 0.2 ml of 40 per cent 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 was indicated by the development of a strong red colour.

3.6 ENUMERATION OF BACTERIA FROM THE ENVIRONMENTAL SAMPLES AND PROCESSING EQUIPMENT

3.6.1 Air

Direct exposure method described by Evancho *et al.* (2001) was employed for the estimation of bacterial count in the air samples of sticking and bleeding area, scalding area and processing hall. To estimate the count in the air, duplicate petri-dishes containing sterile nutrient agar medium were exposed for 15 minutes. The plates were brought to the laboratory in a thermocool container and incubated at 37°C for two days. The number of colonies developed in the duplicate plates was counted and the mean count was expressed as number of colony forming units/ min.

3.6.2 Water

3.6.2.1 Collection of water samples

a) Pond water

Pond water for bacteriological examination was collected following the procedure described by Indian Standards (1978). A clean sterile bottle of 250 ml capacity was held by its bottom and plunged its neck downwards below the surface of the water. The bottle was then turned until the neck pointed slightly

upwards. When the bottle was filled with water, it was raised above the surface of water and the stopper was replaced. The water samples were transported to the laboratory in a thermocool container.

b) Tap water

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

c) Scalding water

The temperature of the scalding tank water was measured during the processing of chicken carcasses. About 200 ml of scalding tank water was collected using a sterile bottle with stopper and allowed to cool at room temperature and brought to the laboratory in a thermocool container.

3.6.2.2 Processing of Water Samples

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

3.6.2.3 Preparation of Water Sample

In order to estimate the bacterial load per ml of water sample, 10 ml was transferred to 90 ml of sterile quarter strength Ringers solution so as to form one in 10 dilution of the sample. Transferring one ml of inoculum into nine ml of the diluent formed further 10 fold dilution. Serial dilution of each sample was made up to 10^7. The selected serial dilution of each sample of water was used to

estimate the total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC) and was carried out as in the case of carcass rinse samples.

3.6.3 Processing Equipment

Swab contact method described by Evancho *et al.* (2001) was followed to collect samples from the eviscerating table, knife and defeathering machine for the estimation of bacterial count.

a. Eviscerating table

To collect samples from the eviscerating table, a sterile swab was moistened with 0.1 per cent sterile peptone water and excess diluent was removed by pressing the swab against the interior wall of the vial with a rotating motion. The swab head was rubbed slowly and thoroughly over 100-cm^2 surface, which was marked with a sterile aluminium template (10 x 10 cm^2 internal area). The swab was rubbed three times, reversing direction between strokes. After swabbing, the head of the swab was cut with a pair of sterile scissors, transferred into a sterile flask containing 0.1 per cent peptone water and brought to the laboratory in a thermocool container

b) Knife

Knife samples were collected with sterile cotton swab as described in the collection of samples from the eviscerating table. The swab was slowly moved firmly three times over the entire surface, reversing the direction each time. After swabbing, the processing and transportation of the swab was carried out as described in the sampling of eviscerating table.

c) Defeathering machine

To collect samples from the defeathering machine the moistened swab was moved over the surface of the defeathering machine several times, reversing

the direction of the swab each time. The swab was processed and transported as in the case of eviscerating table.

Examination of air, water samples, eviscerating table, knife and defeathering machine was repeated at six visits. The preparation, processing and estimation of bacterial load on the swab samples collected from eviscerating table, knife and defeathering machine were carried out as in the case of carcass rinsate.

The bacterial count on the eviscerating table and defeathering machine was expressed as number of colony forming units (cfu) per cm^2 and the count on knife and of water samples was expressed as cfu per ml.

3.7 STATISTICAL ANALYSIS

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 study, 60 dressed chicken carcasses, consisting of 30 each taken after the removal of head and feet (ARHF) and after evisceration (AE) were evaluated for their bacterial quality by estimating the total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC). The carcasses were also examined for the isolation and identification of food-borne pathogens such as salmonellae, *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes.* The samples of air, water, scalding tank water and swab samples from processing equipments such as eviscerating table, knife and steel and defeathering machine were also evaluated to identity the potential source of contamination of chicken carcasses on the dressing line.

4.1 BACTERIAL COUNT

4.1.1 Total Viable Count

The mean total viable count (TVC) of the samples taken after the removal of head and feet (ARHF) and also after evisceration (AE) is given in table 1.

Table 1. Mean total viable count of carcasses taken after the removal of head and feet and after evisceration

Sample		TVC
No.	Step	Mean ± SE (log_{10} cfu/ml)
30	ARHF	$5.88^{*a} \pm 0.13$
30	AE	$4.44^{a} \pm 0.10$

ARHF = after the removal of head and feet AE = after evisceration

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

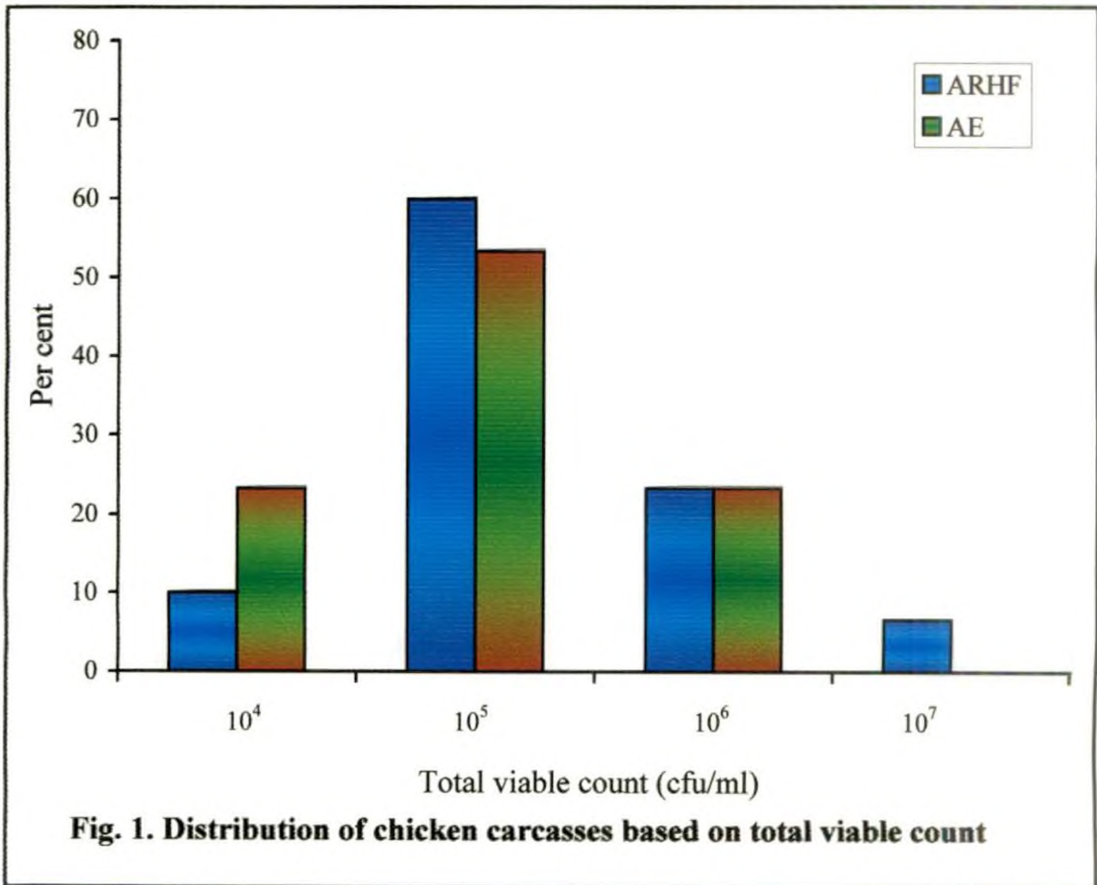


Fig. 1. Distribution of chicken carcasses based on total viable count

Table 3. Mean coliforms count of carcasses taken after the removal of head and feet and after evisceration

Sample		CC
No.	Step	Mean ± SE (log_{10} cfu/ml)
30	ARHF	3.81 ± 0.09
30	AE	3.92 ± 0.12

ARHF = after the removal of head and feet AE = after evisceration.

Analysis of variance test of the data revealed no significant (P> 0.05) difference between the mean coliforms count of the carcasses taken ARHF and AE. However, a higher mean count was observed in the latter group of samples.

4.1.2.1 Distribution of Carcasses Based on Coliforms Count

Distribution of carcasses taken after the removal of head and feet (ARHF) and after evisceration (AE) based on coliforms count is shown in table 4 and illustrate in Fig. 2.

Table 4. Distribution of chicken carcasses based on coliforms count

Sample		CC of carcass (cfu/ml)			
No.	Step	10^2	10^3	10^4	10^5
30	ARHF	1 (33.33)	23 (76.67)	4 (13.33)	2 (6.67)
30	AE	2 (6.67)	16 (53.33)	10 (33.33)	2 (6.67)

ARHF = after the removal of head and feet AE = after evisceration.
Figures in parenthesis indicate per cent.

The carcasses taken ARHF and AE had coliforms count ranging between 10^2 and 10^5 cfu/ml. The count in 6.67 per cent samples each from the former and latter group of carcasses was at the level of 10^5 cfu/ml. In 76.67 per cent samples

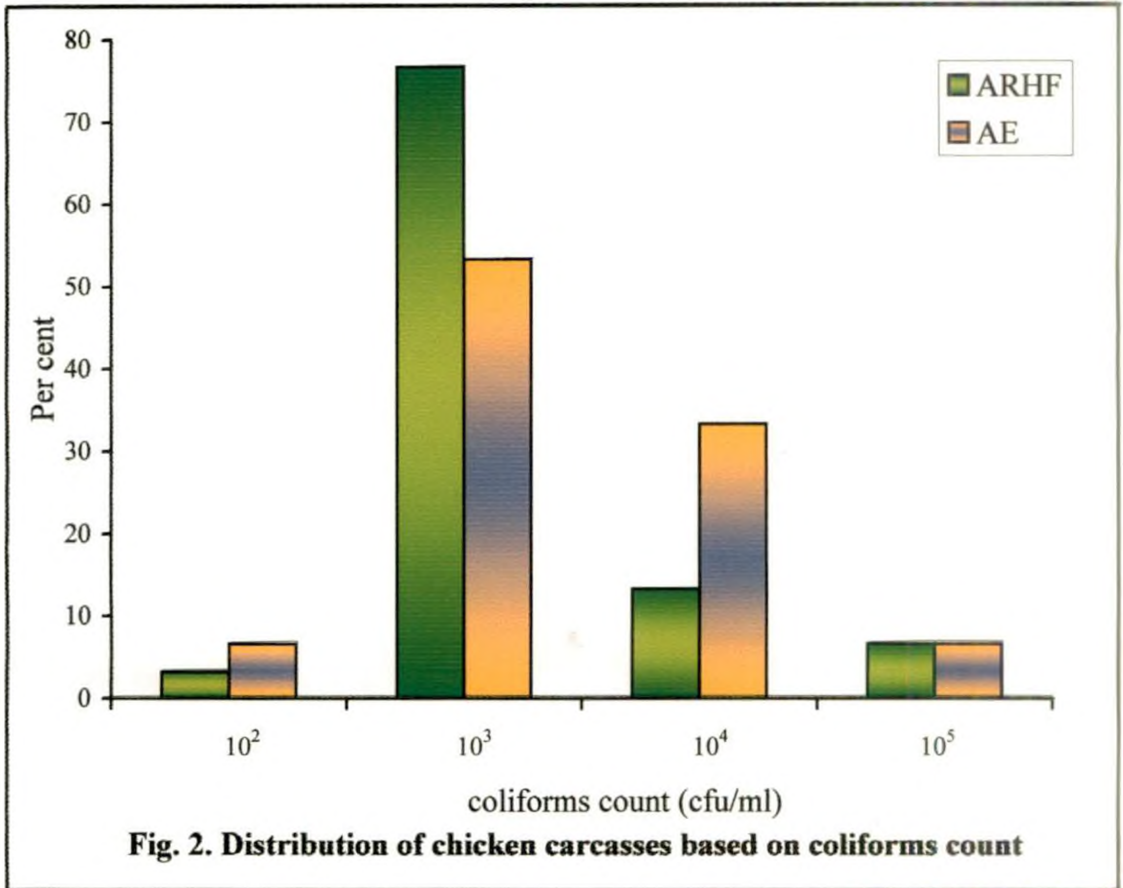


Fig. 2. Distribution of chicken carcasses based on coliforms count

taken ARHF had count at the level of 10^3 cfu/ml, while only 53.33 per cent samples from the other group had count at the above level.

4.1.3 *Escherichia coli* Count

The mean *Escherichia coli* count (ECC) of samples taken to ARHF and AE is given in table 5. Analysis of variance test of the data revealed significant ($P< 0.05$) difference between the mean count of the samples taken after the removal of head and feet (ARHF) and after evisceration (AE). The mean *Escherichia coli* count of the samples from the latter group of carcasses was higher. The organism was detected in 11(36.67 per cent) and 23(76.67 per cent) samples obtained from the carcasses after the removal of head and feet and after evisceration, respectively.

Table 5. Mean *Escherichia coli* count of carcasses taken after the removal of head and feet and after evisceration

Sample		ECC
No.	Step	Mean ± SE (log_{10} cfu/ml)
30	ARHF	$0.89^{a} \pm 0.23$
30	AE	$2.15^{*a} \pm 0.24$

ARHF = after the removal of head and feet AE = after evisceration

$P<0.05$. Figures bearing the same superscript in the same column differ significantly

4.1.3.1 Distribution of Carcasses Based on Escherichia coli Count

The distribution of carcasses based on *Escherichia coli* count is shown in table 6 and illustrated in Fig.3. *Escherichia coli* count of the samples taken from the carcasses ARHF and AE was varied between 10^2 and 10^3 cfu/ml. In 30 per cent of the samples taken from the carcasses after evisceration had the organism

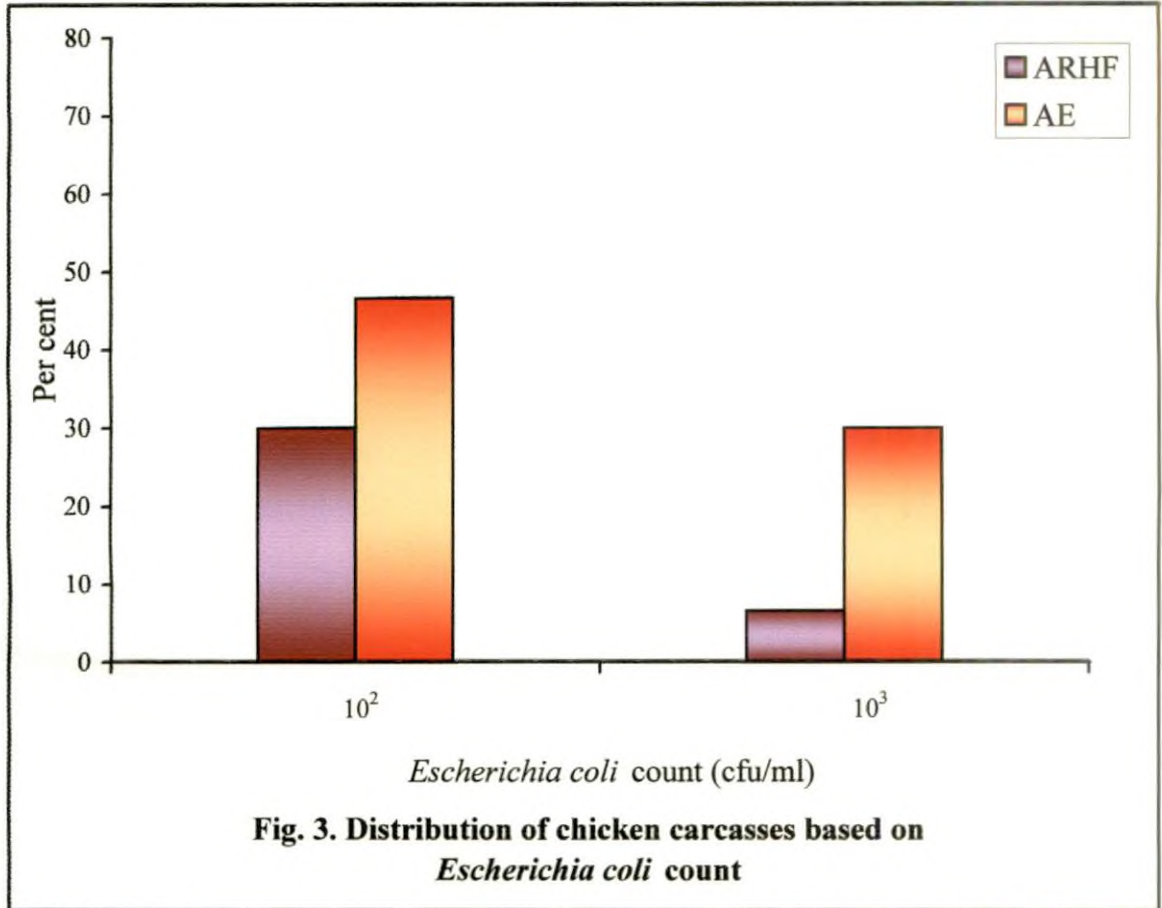


Fig. 3. Distribution of chicken carcasses based on *Escherichia coli* count

at the level of 10^3 cfu/ml but only 6.67 per cent samples belonging to the other group of carcasses revealed the presence of organism at the same level. The count at the level of 10^2 cfu/ml of carcasses rinse was seen in 46.67 per cent of the carcasses belonging to the eviscerated group.

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

Sample		ECC of carcass (cfu/ml)	
No.	Step	10^2	10^3
30	ARHF	9 (30.00)	2 (6.67)
30	AE	14 (46.67)	9 (30.00)

ARHF = after the removal of head and feet AE = after evisceration

Figures in parenthesis indicate per cent.

4.1.4 Faecal Streptococcal Count

The mean faecal streptococcal count of chicken carcass samples obtained from the two sites is given in table 7. All samples collected from both groups of carcasses had faecal streptococcal count, but the mean count of the samples belong to both group did not differ significantly. However, the samples collected from the eviscerated carcasses had a higher mean count per ml of the carcasses rinse.

Table 7. Mean faecal streptococcal count of carcasses taken after the removal of head and feet and after evisceration

Sample		FSC
No.	Step	Mean ± SE ($\log_{10}$ cfu/ml)
30	ARHF	3.89 ± 0.06
30	AE	3.91 ± 0.07

ARHF = after the removal of head and feet AE = after evisceration

4.1.4.1 Distribution of Carcasses Based on Faecal Streptococcal Count

Distribution of chicken carcasses obtained from the two sites, based on faecal streptococcal count is depicted in table 8 and illustrated in Fig.4. The data revealed that the count of the carcasses belonging to both groups varied from 10^3 to 10^4 cfu/ml. In 33.33 per cent samples taken from the carcasses after the removal of head and feet had count at the level of 10^4 cfu/ml of carcass rinse, while the count at the above level was seen in 30 per cent of samples from the carcasses after evisceration. The count in 70 per cent of the latter group of carcasses was at the level of 10^3 cfu/ml.

Table 8. Distribution of chicken carcasses based on faecal streptococcal count

Sample		FSC of carcass (cfu/ml)	
No.	Step	10^3	10^4
30	ARHF	20 (66.67)	10 (33.33)
30	AE	21 (70.00)	9 (30.00)

ARHF = after the removal of head and feet AE = after evisceration

Figures is parenthesis indicate per cent.

4.2 RELATIONSHIP BETWEEN THE BACTERIAL COUNT

All bacterial count were analysed to detect the association between the bacterial count of chicken carcass samples collected after the removal of head and feet and also after evisceration. The correlation coefficient between the mean bacterial count of the samples collected after the removal of head and feet (ARHF) is given in table 9. Analysis of the data revealed a significant ($P<0.05$) and positive correlation between the mean TVC and FSC, CC and ECC, CC and FSC and ECC and FSC. However, a negative and non significant association was observed between the mean TVC and CC and TVC and ECC.

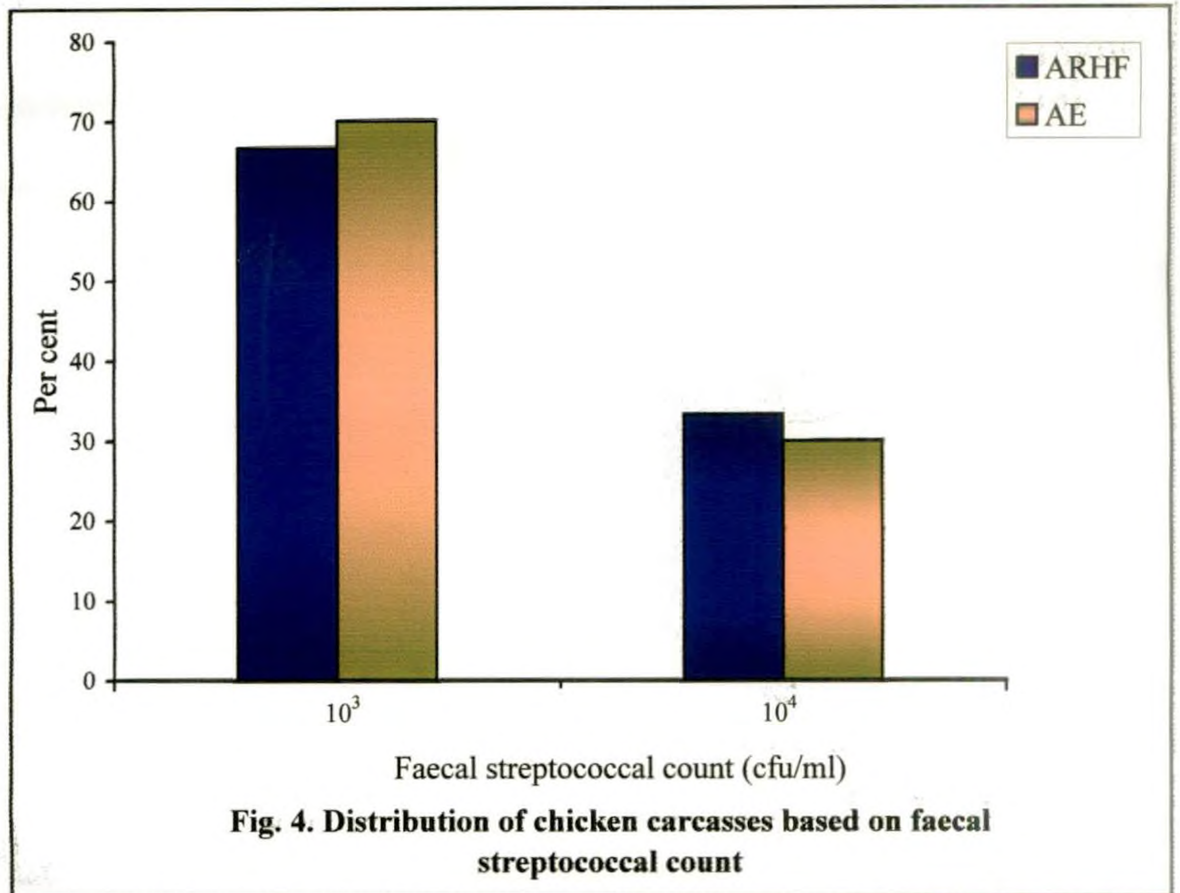


Fig. 4. Distribution of chicken carcasses based on faecal streptococcal count

Table 9. Correlation coefficient between the mean bacterial count of chicken carcass samples collected after the removal of head and feet

Bacterial count	Correlation coefficient between the mean count			
	TVC	CC	ECC	FSC
TVC		-0.07	-0.20	0.44*
CC			0.76*	0.61*
ECC				0.42*

*P< 0.05. TVC – Total viable count, CC – Coliforms count ECC- *Escherichia coli* count, FSC – Faecal streptococcal count.

The relation ship between the mean bacterial count of the samples obtained from the eviscerated carcasses are depicted in table 10. A significant (P<0.05) and positive relationship was observed between the mean CC and ECC and ECC and FSC. The correlation between TVC and CC, TVC and FSC and CC and FSC was positive but non significant. A negative and non significant association existed between the mean TVC and ECC.

Table 10. Correlation coefficient between the mean bacterial count of chicken carcass samples collected after evisceration

Bacterial count	Correlation coefficient between the mean count			
	TVC	CC	ECC	FSC
TVC		0.26	-0.09	0.08
CC			0.40*	0.24
ECC				0.14*

*P<0.05. TVC – Total viable count, CC – Coliforms count, ECC – *Escherichia coli* count, FSC – Faecal streptococcal count.

4.3 ISOLATION AND IDENTIFICATION OF BACTERIA

The carcass rinse obtained from 30 chicken carcasses each taken after the removal of head and feet (ARHF) and after evisceration (AE) was subjected to the isolation and identification of *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Listeria monocytogenes*.

4.3.1 *Escherichia coli*

The investigation revealed that the rinsate prepared from 11 carcasses after the removal of head and feet (ARHF) and 23 carcasses after evisceration (AE) had *Escherichia coli*. All the isolates were identified by the cultural, morphological and biochemical characteristics described by Barrow and Feltham, (1993). All isolates were positive for Eijkman test. The per cent of organism isolated from each group of carcasses is shown in table 11.

Table 11. The per cent of carcasses yielded *Escherichia coli*

Sample		Carcasses	
No.	Step	No. Positive	Per cent
30	ARHF	11	36.67
30	AE	23	76.67

ARHF = after the removal of head and feet AE = after evisceration

A total of 40 *Escherichia coli* isolated from 34 carcasses were serotyped at the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh. The result of serotyping is shown in table 12.

Table 12. Distribution of *Escherichia coli* serotypes

Sl. No.	Serotype	No. of isolates from		Total
		ARHF	AE	
1	O2	-	2	2
2	O5	-	1	1
3	O8	2	1	3
4	O16	-	2	2
5	O21	-	2	2
6	O51	1	-	1
7	O60	2	2	4
8	O64	1	1	2
9	O69	1	-	1
10	O78	-	1	1
11	O81	1	2	3
12	O101	1	2	3
13	O119	1	3	4
14	O123	-	1	1
15	O140	3	4	7
16	Untypable	-	1	1
17	Rough	2	-	2
	Total	15	25	40

ARHF = after the removal of head and feet AE= after evisceration

Of the 40 isolates, 37 belonged to 15 serotypes, two were rough and one was untypable.

The 15 isolates recovered from the carcasses after the removal of head and feet (ARHF) fell into nine serotypes and two of them were rough. The serotypes O51 and O69 were unique for the samples from this group.

A total of 25 *E. coli* were isolated from the eviscerated carcasses and were belonging to 13 serotypes. Only one of the isolates was untypable. The serotypes O2, O5, O16, O21, O78 and O123 were isolated only from these carcasses. However, the serotypes O8, O60, O64, O81, O102, O119 and O140 were isolated from both the groups of carcasses. The serotype O140 was the serotype isolated in highest number from both the groups of carcasses.

4.3.2 *Salmonella*

The carcass rinsate prepared from 30 carcasses each after the removal of head and feet (ARHF) and after evisceration (AE) were subjected to the isolation and identification of *Salmonella.* The details of isolation of *Salmonella* from the carcasses are shown in table 13. A total of three organisms were isolated from the rinsate prepared from three (10 per cent) carcasses after the removal of head and feet and were identified by the cultural, morphological and biochemical characteristics described by Barrow and Feltham, (1993). The isolates were serotyped at the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh.

Table 13. *Salmonella* isolated from chicken carcasses

Sample		No. of positive samples	Serotypes isolated
No.	Step		
30	ARHF	3 (10.00)	*S. enteritidis*
30	AE	Nil	-

ARHF = after the removal of head and feet AE = after evisceration

Figures in parenthesis indicate per cent

Salmonella enteritidis was isolated from 10 per cent of the samples taken from the carcasses after the removal of head and feet (ARHF) but none of the samples from the eviscerated carcasses revealed the presence of the organism.

4.3.3 *Staphylococcus aureus*

All rinse samples prepared from the carcasses after the removal of head and feet and also from the eviscerated carcasses were tested for the isolation and identification of *Staphylococcus aureus.* The number of organisms isolated and identified from each group of carcasses are shown in table 14. All isolates were identified by the cultural, morphological and biochemical characteristics described by Barrow and Feltham (1993). The organism was isolated from 11(36.67 per cent) rinsate samples collected from the eviscerated carcasses. None of the samples collected from the carcasses after the removal of head and feet (ARHF) yielded *Staphylococcus aureus.*

Table 14. *Staphylococcus aureus* isolated from chicken carcasses

Sample		No. of positive samples	Per cent
No.	Step		
30	ARHF	Nil	-
30	AE	11	36.67

ARHF = after the removal of head and feet AE = after evisceration

4.3.4 Listeria monocytogenes

None of the samples from the carcasses after the removal of head and feet (ARHF) and also after evisceration (AE) revealed the presence of *Listeria monocytogenes.*

4.4 BACTERIAL COUNT FROM THE ENVIRONMENTAL SAMPLES AND PROCESSING EQUIPMENT

4.4.1 Air

The mean bacterial load of air samples from the sticking and bleeding area, scalding area and processing hall is given in table 15. Air samples from the

processing hall had a high mean total viable count and the lowest count of the organism was observed in the samples collected from the scalding area.

Table 15. Mean total viable count of air samples from three different sites on the processing line

Sl. No.	Sampling site	TVC (Mean ± SE log_{10} cfu/min)
1.	Sticking and bleeding area	1.63 ± 0.98
2.	Scalding area	1.54 ± 0.01
3.	Processing hall	1.99 ± 0.83

4.4.2 Processing Equipment and Water Samples

The mean bacterial load of various processing equipments and water samples is shown in table 16. All samples collected from these sources were tested to detect the level of TVC, CC, ECC and FSC. The water samples collected from the scalding tank revealed all the above bacteria but the samples collected from defeathered carcass wash, after washing the carcasses, pond and tap did not reveal the presence of *Escherichia coli* and feacal streptococci.

The scalding tank water had the highest total viable count and the lowest count was observed in the tap water sample.

Among the equipment, samples collected from eviscerating table had revealed the presence of total viable bacteria, coliforms and faecal streptococci but the samples obtained from other sources showed the presence of only total viable bacteria and coliforms. The samples taken from defeathering machine had the highest mean total viable count and the knife samples had the lowest mean count. The highest number of coliforms organisms were detected in the samples

obtained from the defeathering machine followed by the samples collected from eviscerating table and knife swab.

Table 16. The mean bacterial load of various processing equipment and water samples

Source of sample	Bacterial count (Mean ± SE, $\log_{10}$ cfu/cm^2 or/ml)			
	TVC	CC	ECC	FSC
Water samples				
a) Pond water	5.10 ± 0.79	1.39 ± 0.77	-	-
b) Tap water	2.89 ± 0.12	0.45 ± 0.15	-	-
c) Scalding tank water (post treatment)	6.94 ± 0.37	2.69 ± 0.18	1.3 ± 0.30	3.03 ± 0.21
d) Defeathered carcass wash	4.50 ± 0.15	-	-	-
e) Final carcass wash	3.95 ± 0.22	-	-	-
Equipment				
a) Defeathering machine	5.98 ± 0.52	2.51 ± 0.25	-	-
b) Knife	2.75 ± 0.32	1.50 ± 0.23	-	-
c) Eviscerating table	4.95 ± 0.22	1.75 ± 0.23	-	1.20 ± 0.14

TVC = Total viable count, CC = Coliforms count, ECC = *Escherichia coli* count, FSC = Faecal streptococcal count

Discussion

5. DISCUSSION

In the present study, a total of 60 chicken carcasses, 30 each, from two different steps on the dressing lines viz., after the removal of head and feet (ARHF) and also after evisceration (AE) were evaluated for their bacterial quality by estimating the various bacterial counts and also to detect certain bacteria of public health significance. The environmental and processing equipment samples were also tested to detect the level and type of bacterial organisms present in these samples and to identify their role in contamination of chicken carcasses.

5.1 BACTERIAL COUNT

All carcass rinse samples were tested to detect the bacterial load per ml of carcass rinse by estimating the total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC).

5.1.1 Total Viable Count

Analysis of variance test of the data revealed significant ($P<0.05$) difference between the mean count of the samples taken after the removal of head and feet (ARHF) and after evisceration (AE) of the carcass. The former group of carcass had a higher mean count (5.88 ± 0.13 $\log_{10}$ cfu/ml) and was one log greater than that reported by Berrang *et al.* (2000) and Fluckey *et al.* (2003). However the count in the latter group of carcass 4.44 ± 0.10 $\log_{10}$ cfu/ml and was also one log greater than that reported by Fluckey *et al.* (2003).

In the case of samples taken ARHF, only two per cent had count at the level of 10^7 cfu/ml. The count of all the samples from both groups was within the limit recommended by ICMSF (1986) (Gracey *et al.*, 1999).

Total viable count is used as an index of sanitary quality and handling history of foods (Jay, 1978). The high count in carcasses after the removal of

head and feet (ARHF) is an indication of poor hygienic practices followed during dressing. Reduction of the count in the carcasses after evisceration (AE) could be attributed to thorough washing and immersion in chilled water.

5.1.2 Coliforms Count

The samples taken after evisceration (AE) had a higher mean coliforms count, $3.92 \pm 0.12 \log_{10}$ cfu/ml, as compared to the count of the carcasses after removal of head and feet, $3.81 \pm 0.09 \log_{10}$ cfu/ml. The latter count was greater than that reported by Berrang *et al.* (2000) and Fluckey *et al.* (2003). The count on eviscerated carcasses of the present study was higher than that reported by Fluckey *et al.* (2003) who had recorded the count as $3.27 \log_{10}$ cfu/ml. Coliforms count was observed at the level of 10^5 cfu/ml, in two per cent of the carcasses in both groups. The count in 76.67 per cent of the ARHF and 53.33 per cent of the AE carcasses was at the level of 10^3 cfu/ml. Coliforms may be faecal or non-faecal in origin. The organisms are generally accepted as an index of faecal pollution. The high count on carcasses taken after evisceration might be due to the contamination of the carcasses from the intestinal content and cross contamination from the eviscerating table. Coliforms count is used to assess the overall quality and hygienic condition prevailing during the processing of food (Kornacki and Johnson, 2001).

5.1.3 *Escherichia coli* Count

Analysis of variance test of the data revealed significant ($P<0.05$) difference between the mean *Escherichia coli* count of samples taken from the carcasses, ARHF and AE. The organism could not be detected in 67 per cent of the samples from the former group and 23 per cent of the samples from the latter group. The mean *Escherichia coli* count of the carcasses taken ARHF was two log lower than that reported by Berrang *et al.* (2000) and three log lower than that recorded by Fluckey *et al.* (2003). The count of eviscerated carcasses of the

present investigation was 0.93 $\log_{10}$ cfu/ml, lower than that reported by the latter workers.

The count at the level of 10^3 cfu/ml was observed in 6.67 per cent and 30.00 per cent of the ARHF and AE carcasses, respectively. However, 30.00 and 46.67 per cent of the samples from ARHF and AE, respectively had the count at the level of 10^2 cfu/ml.

The high count of the organism on the eviscerated carcasses might be attributed to the contamination with intestinal content of chicken, since the organism is found at high level in the intestinal tract of broiler chicken (Vorster *et al.*, 1994). It may also be due to careless evisceration (Pattnaik *et al.*, 1997). The contamination of carcass might have also occurred from contaminated water and the personnel engaged in various dressing processes.

5.1.4 Faecal Streptococcal Count

All samples taken after the removal of head and feet (ARHF) and after evisceration (AE) had faecal streptococci, but the mean count of the samples belonging to both groups did not differ significantly. However, the samples collected from the eviscerated carcasses had a higher mean count (3.91 ± 0.07 $\log_{10}$ cfu/ml).

The count in 66.67 per cent and 70.00 per cent of carcasses belonging to ARHF and AE, respectively, was at the level of 10^3 cfu/ml. However a count of 10^4 cfu/ml was also observed in 33.33 and 30.00 per cent of the samples, respectively, from ARHF and AE. Faecal streptococci are normally present in mammalian faeces and act as indicators of poor factory sanitation (Brown and Baird-Parker, 1982). The high count of the organism in eviscerated carcasses indicate poor evisceration technique.

5.2 RELATIONSHIP BETWEEN BACTERIAL COUNT

5.2.1 After the Removal of Head and Feet

A positive and significant ($P<0.05$) correlation was observed between the mean TVC and FSC, CC and ECC, CC and FSC and ECC and FSC. These findings suggest that an increase in FSC on carcass surface leads to a corresponding increase in TVC, CC and ECC and the increase in ECC leads to a corresponding increase in CC. Since faecal streptococci and *Escherichia coli* are of intestinal origin, an increase in these organisms with a corresponding increase in CC indicate that majority of the coliforms are of intestinal origin.

5.2.2 After Evisceration

Analysis of the data revealed a significant ($P<0.05$) and positive relationship between the mean CC and ECC and ECC and FSC. This indicates that the major contamination of the carcasses was of intestinal origin. The association between the mean TVC and FSC and CC and FSC was positive but non significant. It was also observed that a negative and non-significant association existed, between the mean TVC and ECC.

5.3 ISOLATION OF BACTERIA

Samples collected from the carcasses after the removal of head and feet and after evisceration were examined to isolate and identify *Escherichia coli*, salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes*.

5.3.1 *Escherichia coli*

Escherichia coli is an indicator and pathogenic organism found both in human and animal intestines and its presence on carcasses indicates the contamination of the carcasses with the excreta and/or with the contaminated environmental sources like water. Thus, the presence of the organism in the meat indicates unsatisfactory hygienic practices followed during the dressing of

poultry. Certain strains of *Escherichia coli* can cause enteric disease in man, either by elaborating an enterotoxin or by penetrating the intestinal epithelium. Enteric illness associated with the consumption of food or water containing *Escherichia coli* has been suspected for the last many years.

In the present study, *Escherichia coli* was isolated from 36.67 per cent of carcasses taken after the removal of head and feet and 76.67 per cent of eviscerated carcasses. Thirty seven of the 40 isolates were grouped under 15 serotypes, and two were rough and one was untypable. Of 15 serotypes, seven were found to be pathogenic which included O2, O5, O8, O56, O78, O101 and O119.

The serotypes O8, O78 and O101 were categorized as Enterotoxigenic *Escherichia coli* (ETEC) which are associated with traveller's diarrhoea and cholera like disease in children under five years of age (Smith and Cheasty, 1998). The serotype O8 was isolated from two samples taken from ARHF and one sample taken from AE, while O78 was isolated only from one sample of the eviscerated carcasses. Nair *et al.* (1990) reported the isolation of O78 from dressed chicken procured from the market. Raj (2002) also isolated the serotypes O8 and O78 from dressed broiler chicken. One sample taken after the removal of head and feet and two samples of eviscerated carcasses revealed the presence of O101 serotype.

Enterohaemorrhagic *Escherichia coli* (EHEC) or the Verotoxigenic *Escherichia coli* (VTEC) organisms isolated from the samples included serotypes O2 and O5. Both the serotypes were isolated only from the eviscerated carcasses. Infection with VTEC in man causes diarrhoea, haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) (Smith and Cheasty, 1998). These strains can produce a toxin similar to that produced by *Shigella dysenteriae* and hence also called shiga like toxin producing *Escherichia coli* (SLTEC) Samadpour *et al.* (1994) reported the isolation of shiga like toxin producing *Escherichia coli* from 12 per cent of chicken samples. The isolation of serotype

O5 was reported from chicken meat (Nair *et al.*, 1990 and Sharma *et al.*, 1995) and also from dressed broiler chicken by Raj (2002).

The serotype O119 isolated from both groups of carcasses is categorized as Enteropathogenic *Escherichia coli* (EPEC). Infection with EPEC usually causes a syndrome of watery diarrhoea, vomiting and fever in infants and children (Smith and Cheasty, 1998). The serotype O51 obtained from the carcasses taken after the removal of head and feet comes under the group of Enteroaggregative *Escherichia coli* (EAggEC). In developing countries infection with EAggEC is associated with persistent diarrhoea (Smith and Cheasty, 1998). The same serotype was also isolated by Nair *et al.* (1990) from the dressed broiler birds procured from the poultry processing unit at CFTRI.

The isolation of ETEC, EHEC, EPEC and EAggEC from poultry carcass rinses in the present study indicates the importance of poultry meat in causing food borne infections. The presence of various serotypes even in the eviscerated and finally washed carcasses indicates that hygienic practices followed during processing and handling are not satisfactory.

5.3.2 *Salmonella*

Salmonella enteritidis was isolated from three (10 per cent) carcass rinse samples obtained after the removal of head and feet but none of the samples from the eviscerated carcasses revealed the presence of the organism. The isolation of the serotype from raw chicken meat was reported by Jerngklinchan *et al.* (1994), from imported poultry meat by Telo *et al.* (1998) and Duffy *et al.* (1999), from poultry carcasses by Hang'Ombe *et al.* (1999), Peresi *et al.* (1999) and Uyttendaele *et al.* (1999) and from retail broiler chicken samples by Chang (2000). 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.

Salmonellosis is the most prevalent food-borne disease in many countries of the world (Chang, 2000). 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 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 in human beings (Zivkovic *et al.*, 1997).

5.3.3 *Staphylococcus aureus*

Eleven (36.67 per cent) rinsate samples collected from eviscerated carcasses revealed the presence of *Staphylococcus aureus* but none of the samples collected from the carcasses after the removal of head and feet yielded the organism. The isolation of the organism from chicken meat samples was also reported by Nkanga and Uraih (1981), Murugkar *et al.* (1993), Vorster *et al.* (1994), Pattnaik *et al.* (1997), Capita *et al.* (2001) and Soriano *et al.* (2001).

Staphylococci are part of the normal flora of animals and man. Because of this ubiquitous occurrence in nature they are often found in various raw meats. *Staphylococcus aureus* is frequently associated with food poisoning (Vorster *et al.*, 1994). The presence of the organism in the finally washed and eviscerated carcasses indicates that hygienic practices followed during processing are not satisfactory. So attention must be paid to sanitation and personnel hygiene to minimize the contamination of the carcasses with the organism.

5.3.4 *Listeria monocytogenes*

All the carcasses from both the groups were found free of *Listeria monocytogenes.*

5.4 BACTERIAL QUALITY OF ENVIRONMENTAL SAMPLES

The mean total viable counts of air samples from the area of sticking and bleeding, scalding and processing hall were 1.63 ± 0.98, 1.54 ± 0.01 and 1.99 ±

0.83 $\log_{10}$ cfu/min, respectively. The count obtained in the present study was higher than that reported by Eisel *et al.* (1997) from a red meat processing plant and almost similar to that obtained by Rahkio and Korkeala (1997). The study reveals that air borne bacteria have a significant contribution in the contamination of chicken carcasses during the various stages of processing.

The mean total viable counts of water samples from pond, tap, scalding tank, defeathered carcass wash and final carcass wash were 5.10 ± 0.79, 2.89 ± 0.12, 6.94 ± 0.37, 4.50 ± 0.15 and 3.95 ± 0.22 $\log_{10}$ cfu/ml, respectively. Water samples from pond, tap and scalding tank revealed the presence of coliforms and the mean counts were 1.39 ± 0.77, 0.45 ± 0.15 and 2.69 ± 0.18 $\log_{10}$ cfu/ml, respectively. *Escherichia coli* and faecal streptococci were detected only in scalding tank water and the corresponding counts were 1.30 ± 0.30 and 3.03 ± 0.21 $\log_{10}$ cfu/ml. The total viable count of the water samples was similar to that reported by Tarwate *et al.* (1993) and Abu-Ruwaida *et al.* (1994). Coliforms count reported by Tarwate *et al.* (1993) was four to six log higher than the results of the present study. The total viable count of the scalding tank water was similar to that reported by Anand *et al.* (1989) and Lillard (1990). Water used in abattoir should be of potable quality (Gracey *et al.*, 1999). The findings show that the water used in the abattoir was far away from international drinking water quality standards prescribed by W.H.O. (Rajvaidya and Markandey, 1998). The high count in the water samples indicates its unwholesomeness and its role in the contamination of carcasses especially during washing.

The mean total viable count of processing equipments such as defeathering machine, knife and eviscerating table ranged from 2.75 ± 0.32 to 5.98 ± 0.52 $\log_{10}$ cfu/cm^2 or per ml and it was four log lower than that reported by Tarwate *et al.* (1993). The coliforms count varied between 1.50 ± 0.23 and 2.51 ± 0.25 $\log_{10}$ cfu/cm^2 or per ml. Faecal streptococci were detected only in the samples taken from the eviscerating table and the mean count was 1.20 ± 0.14 $\log_{10}$ cfu/cm^2.

Summary

6. SUMMARY

The consumer safety and shelf life of chicken carcasses and its products are influenced by their bacterial quality. During the present investigation, a total of 60 chicken carcass rinses, consisting of 30 each taken after the removal of head and feet (ARHF) and after evisceration (AE) were evaluated for their bacterial quality by estimating total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC). All carcass rinses were also subjected to isolation and identification of food borne pathogens such as *Escherichia coli*, Salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes*. The role of carcass dressing and environment and equipment in the contamination of chicken carcasses were also evaluated.

All bacterial count were subjected to statistical analysis. Analysis of variance test of the data revealed significant ($P<0.05$) difference between the mean total viable count of samples taken ARHF and AE. The samples taken ARHF had a high mean count ($5.88 \pm 0.13 \log_{10}$ cfu/ml) compared to the samples taken from carcasses AE. The count on carcasses ARHF ranges from 10^4 to 10^7 cfu/ml while the count on eviscerated carcasses vary between 10^4 and 10^6 cfu/ml. The study also revealed that 60 per cent of the carcasses from the former group and 53.33 per cent of carcasses from the latter group had count at the level of 10^5 cfu/ml. Only two per cent of carcasses taken after the removal of head and feet had count at the level of 10^7 cfu/ml.

The samples taken from eviscerated carcasses had a high mean coliforms count ($3.92 \pm 0.12 \log_{10}$ cfu/ml) but the counts did not differ significantly between the groups. The count of both groups of carcasses ranged between 10^2 and 10^5 cfu/ml. The count in 76.67 per cent samples from the carcasses ARHF and 53.33 per cent of samples belonging to carcasses after evisceration was at the level of 10^3 cfu/ml.

Analysis of variance test of *Escherichia coli* count (ECC) of the samples revealed significant (P<0.05) difference between the mean count of the samples from the carcasses ARHF and AE. The mean count of the samples from the latter group of carcasses was high. The count of the samples from the carcasses ARHF and AE was varied between 10^2 and 10^3 cfu/ml. The count at the level of 10^3 cfu/ml was observed in 6.67 per cent of the carcasses from the former group and 30.00 per cent of the carcasses from the latter group.

All samples collected from carcasses ARHF and AE had faecal streptococci but the mean count of the samples belong to both groups did not differ significantly. However, the count of the samples collected from the eviscerated carcasses was high. The study revealed that the count of the carcasses belonging to both groups ranged between 10^3 and10^4 cfu/ml. In 66.67 per cent samples taken from the carcasses ARHF and 70.00 per cent samples taken from the carcasses AE had count at the level of 10^3 cfu/ml.

The correlation coefficient between the various count was determined. A significant (P<0.05) and positive relationship was observed between the mean CC and ECC and ECC and FSC of the samples taken from the carcasses AE. The correlation between the mean TVC and FSC and CC and FSC is positive but non significant. A negative and non significant association was existed between the mean TVC and ECC.

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

Escherichia coli was isolated from 11 (36.67 per cent) and 23 (76.67 per cent) samples taken from the carcasses belonging to ARHF and AE, respectively. From these samples, 40 *Escherichia coli* isolates were obtained and were

serotyped at the National Salmonella and Escherichia centre (NSEC), Central Research Institute, Kasauli, Himachal Pradesh. The isolates belonged to 15 serotypes, two were rough and one was untypable. The 15 serotypes consisted of O2, O5, O8, O16, O21, O51, O60, O64, O69, O78, O81, O101, O119, O123 and O140.

The serotypes O2 and O5 were categorized as Entrohaemorrhagic *Escherichia coli* (EHEC) and O8, O78 and O101 in Enteroxigenic *Escherichia coli* (ETEC). The serotype O119 was Enteropathogenic *Escherichia coli* (EPEC) and O51 belonged to Enteroaggregative *Escherichia coli* (EaggEC).

A total of four salmonellae were isolated from the rinsate prepared from three (10 per cent) carcasses after the removal of head and feet. The isolates were serotyped as *Salmonella enteritidis* at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh. None of the samples from the eviscerated carcasses revealed the presence of *Salmonella*.

Staphylococcus aureus was isolated from 11 (36.67 per cent) rinsate samples collected from eviscerated carcasses but none of the samples from the carcasses after the removal of head and feet yielded the organism.

None of the samples from the carcasses after the removal of head and feet (ARHF) and also after evisceration (AE) revealed the presence of *Listeria monocytogenes*.

The mean total viable count of air samples from three different sites on the processing line was determined. The samples from the processing hall had a high mean count (1.99 ± 0.83 $\log_{10}$ cfu/min) followed by sticking and bleeding area (1.63 ± 0.98 $\log_{10}$ cfu/min) and scalding area (1.54 ± 0.01 $\log_{10}$ cfu/min).

The water samples from the pond, tap, scalding tank, defeathered carcass wash, final carcass wash and samples from the processing equipments were evaluated to detect the level of total viable bacteria, coliforms, *Escherichia coli*

and faecal streptococci. The water samples from the scalding tank revealed the presence of all the above bacteria but pond and tap water samples, defeathered carcass wash and final carcass wash did not reveal the presence of *Escherichia coli* and faecal streptococci. Highest total viable count was obtained in the samples collected from the scalding tanks and lowest count for the tap water samples.

Among the equipments, samples from the defeathering machine had TVC, CC and FSC but the samples from the other sources had only TVC and CC. The highest mean count was obtained for the samples taken from the defeathering machine, followed by eviscerating table and knife swab.

References

REFERENCES

Abu-Ruwaida, A.S., Sawaya, W.N., Dashti, B.H., Murad, M. and Al-Othman, H.A. 1994. Microbiological quality of broilers during processing in a modern commercial slaughter house in Kuwait. *J. Fd. Prot.* **57**(10): 887-892

Al-Hindawi, N. and Rished, R. 1979. Presence and distribution of *Salmonella* species in some local foods from Baghdad City, Iraq. *J. Fd. Prot.* **42**(11): 877-880

Al-Rajab, W.J. and Hussain, F.M. 1982. *Salmonella* serotypes isolated from food samples in Iraq. *Indian J. Comp. Microbiol. Immunol. Inf. Dis.* **3**(1): 1-6

Anand, S.K., Mahapatra, C.M., Pandey, N.K. and Verma, S.S. 1989. Microbiological changes on chicken carcasses during processing. *Indian J. Poult. Sci.* **24**(3): 203-209

Anand, S.K., Pandey, N.K., Mahapatra, C.M. and Verma, S.S. 1990. Microbiological profile of chicken sausages during preparation and storage. *Indian J. Poult. Sci.* **25**(2): 97-102

Anand, S.K., Pandey, N.K., Mahapatra, C.M. and Verma, S.S. 1991. Microbiological quality and shelf life of chicken patties stored at -18°C. *Indian J. Poult. Sci.* **26**(2): 105-108

Anand, S.K., Pandey, N.K., Mahapatra, C.M. and Verma, S.S. 1992. Changes in microflora during preparation and storage of chicken tandoori. *Indian J. Poult. Sci.* **27**(1): 51-53

Anand, S.K., Verma, S.S., Pandey, N.K. and Mahapatra, C.M. 1987. A note on incidence of microflora on freshly dressed poultry. *Indian J. Poult. Sci.* **22**(1): 54-55

Andrews, W.H., Flowers, R.S., Silliker, J. and Bailey, J.S. 2001. *Salmonella. Compendium of Methods for the Microbiological Examination of Foods.* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington, pp. 357-380

Antunes, P., Reu, C., Sousa, J.C., Pestana, N. and Peixe, L. 2002. Incidence and susceptibility to antimicrobial agents of *Listeria* spp. and *Listeria monocytogenes* isolated from poultry carcasses in Porto, Portugal. *J. Fd. Prot.* **65**(12): 1888-1893

*Archile, C.A.C., De Martinez, B.Y.B., Corser, P.I. and Salas, M.E.J. 1992. Chemical and microbiological composition of mechanically deboned poultry meat. *Revista Cieutifica* **9**(4): 276-281

*Bailey, J.S., Fletcher, D.L. and Cox, N.A. 1989. Recovery and serotype distribution of *Listeria monocytogenes* from broiler chickens in South Eastern United States. *J. Fd. Prot.* **52**(3): 148-150

Bailey, J.S., Lyon, B.G., Lyon, C.E. and Windham, W.R. 2000. The microbiological profile of chilled and frozen chicken. *J. Fd. Prot.* **63**(9): 1228-1230

Banerjee, R., Kapoor, K.N., Agarwal, R.K. and Ghatak, S. 2001. Verotoxin-producing *Escherichia coli* (VTEC) in foods of animal origin. *J. Fd. Sci. Technol.* **38**(1): 82-84

Barrow, C.J. and Feltham, R.K.A. 1993. *Cowan and Steel's Manual for the Identification of Medical Bacteria.* Third edition. Cambridge University Press, London. p. 238

Berrang, M.E., Dickens, J.A. and Musgrovet, M.T. 2000. Effects of hot water application after defeathering on the levels of *Campylobacter*, coliforms bacteria and *Escherichia coli* on broiler carcasses. *Poult. Sci.* **79**: 1689-1693

Berrang, M.E., Ladely, S.R. and Buhr, R.J. 2001. Presence and level of *Campylobacter*, coliforms, *Escherichia coli* and total aerobic bacteria recovered from broiler parts with and without skin. *J. Fd. Prot.* **64**(2): 184-188

Boer, E.D. and Hahne, M. 1990. Cross-contamination with *Campylobacter jejuni* and *Salmonella* spp. from raw chicken products during food preparation. *J. Fd. Prot.* **53**(12): 1067-1068

Bok, H.E., Holzapfel, W.H., Odendaal, E.S. and van der Linde, H.J. 1986. Incidence of food borne pathogens on retail broilers. *Int. J. Fd. Microbiol.* **3**: 273-285

Brown, M.H. and Baird-Parker, A.C. 1982. The microbiological examination of meat. *Meat Microbiology.* (ed. Brown, M.H.). Applied Science Publishers, England, pp. 423-520

Campbell, D.F., Johnston, R.W., Campbell, G.S., Mc Clain D. and Macaluso, J.F. 1983. The microbiology of raw, eviscerated chickens: a ten year comparison. *Poult. Sci.* **62**: 437-444

Capita, R., Calleja, C.A., Fernandez, M.D.C.G. and Moreno, B. 2001. Microbiological quality of retail poultry carcasses in Spain. *J. Fd. Prot.* **64**(12): 1961-1966

Carl, K.E. 1975. Oregon's experience with microbiological standards for meat. *J. Milk Fd. Technol.* 38: 483-486

Cason, J.A., Bailey, J.S., Stern, N.J., Whittemore, A.D. and Cox, N.A. 1997. Relationship between aerobic bacteria, salmonellae and *Campylobacter* on broiler carcasses. *Poult. Sci.* **76**(7): 1037-1041

Chang, Y.H. 2000. Prevalence of *Salmonella* spp. in poultry broilers and shell eggs in Korea. *J. Fd. Prot.* **63**(5): 655-658

Choi, Y., Cho, S., Park, B., Chung, D. and Oh, D. 2001. Incidence and characterization of *Listeria* spp. from foods available in Korea. *J. Fd. Prot.* **64**(4): 554-558

Cox, N.A., Bailey, J.S., Lyon, C.E., Thomson, J.E. and Hudspeth, J.P. 1983. Microbiological profile of chicken patty products containing broiler giblets. *Poult. Sci.* **62**: 960-964

Cox, N.A., Thomson, J.E. and Bailey, J.S. 1981. Sampling of broiler carcasses for *Salmonella* with low volume water rinse. *Poult. Sci.* **60**: 768-770

Doyle, M.P. and Schoeni, J.L. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. *Appl. Environ. Microbiol.* **53**(10): 2394-2396

Duffy, G., Cloak, O.M., O'Sullivan, M.G., Guillet, A., Sheridan, J.J., Blair, I.S. and McDowell, D.A. 1999. The incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. *Fd. Microbiol.* **16**: 623-631

Edwards, P.R. and Ewing, W.H. 1972. *Identification of Enterobacteriaceae*. Third edition. Burgess Publishing Company, p. 356

Eisel, W.G., Linton, R.H. and Muriana, P.M. 1997. A survey of microbial levels for incoming raw beef, environmental sources and ground beef in a red meat processing plant. *Fd. Microbiol.* **14**: 273-282

Evancho, G.M., Sveum, W.H. Moberg, L.J. and Frank, J.F. 2001. Microbiological monitoring of the food processing environment. *Compendium of Methods for the Microbiological Examination of Foods.* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington, pp. 25-35

Fliss, I., Simard, R.E. and Ettriki, A. 1991. Microbiological quality of different fresh meat species in Tunisian slaughter houses and markets. *J. Fd. Prot.* **54**(10): 773-777

Fluckey, W.M., Sanchez, M.X., McKee, S.R., Smith, D., Pendleton, E. and Brashears, M.M. 2003. Establishment of a microbiological profile for an air-chilling poultry operation in the United States. *J. Fd. Prot.* **66**(2): 272-279

*Genigeorgis, C.A., Dutulescu, D. and Garayzabal, F.J. 1989. Prevalence of *Listeria* spp. in poultry meat at the supermarket and slaughter house level. *J. Fd. Prot.* **52**(9): 618-624

Genigeorgis, C.A., Oanca, P. and Dutulescu, D. 1990. Prevalence of *Listeria* spp. in turkey meat at the supermarket and slaughterhouse level. *J. Fd. Prot.* **53**(4): 282-288

Geornaras, I., de Jesus, A.E., van Zyl, E. and von Holy, A. 1996. Bacterial populations associated with poultry processing in a South African abattoir. *Fd. Microbiol.* **13**: 457-465

Gill, C.O., Baker, L.P. and Jones, T. 1999. Identification of inadequately cleaned equipment used in a sheep carcass-breaking process. *J. Fd. Prot.* **62**(6): 637-643

Gracey, J., Collins, D.S. and Huey, R. 1999. *Meat Hygiene.* Tenth edition. W.B. Saunders Company, London, p. 758

Hang'Ombe, B.M., Sharma, N.R., Skjerve, E. and Tuchil, L.M. 1999. Isolation of bacteria during processing of chicken carcasses for the market in Lusaka, Zambia. *Vet. Arhiv.* **69**(4): 191-197

Harrison, W.A., Griffith, C.J., Tennant, D. and Peters, A.C. 2001. Incidence of *Campylobacter* and *Salmonella* isolated from retail chicken and associated packaging in South Wales. *Lett. Appl. Microbiol.* **33**:450-454

Indian Standards. 1978. IS:1622-1964. *Methods of Sampling and Test for Microbiological Examination of Water Used in Industry.* Indian Standards Institution, Manak Bhavan, 9, Bhadur Shah Zafar Marg, New Delhi – 1

Indian standards. 1980. SP:18 *Handbook of Food Analysis. Part I. General Methods.* Indian Standards Institution. Manak Bhavan, 9, Bhadur Shah Zafar Marg, New Delhi –1.

Izat, A.L., Kopek, J.M. and McGinnis, J.D. 1991. Incidence, number and serotypes of *Salmonella* on frozen broiler chickens at retail. *Poult. Sci.* **70**: 1438-1440

James, W.O., Williams, W.O., Prucha, J.C., Johnston, R. and Christensen, W. 1992. Profile of selected bacterial counts and *Salmonella* prevalence on raw poultry in a poultry slaughter establishment. *J. Am. Vet. Med. Assoc.* **200**(1): 57-59

Janky, D.M., Arafa, A.S., Oblinger, J.L., Koburger, J.A. and Fletcher, D.L. 1978. Sensory, physical and microbiological comparison of brine-chilled, water-chilled and hot-packaged (no chill) broilers. *Poult. Sci.* **57**: 417-421

Jay, J.M. 1978. *Modern Food Microbiology.* Second edition. D. Van Nostrand Company, New York, p. 479

Jayaraman, M.S., Venugopalan, A.T. and Padmanabhan, V.D. 1972. *Salmonella* serotypes from animals and poultry meat. *Cheiron* **1**(1): 36-41

Jerngklinchan, J., Koowatananukul, C., Daengprom, K. and Saitanu, K. 1994. Occurrence of salmonellae in raw broilers and their products in Thailand. *J. Fd. Prot.* **57**(9): 808-810

Jetton, J.P., Bilgili, S.F., Conner, D.E., Kotrola, J.S. and Reiber, M.A. 1992. Recovery of salmonellae from chilled broiler carcasses as affected by rinse media and enumeration method. *J. Fd. Prot.* **55**(5): 329-332

Johansson, T. 1998. Enhanced detection and enumeration of *Listeria monocytogenes* from food stuffs and food-processing environments. *Int. J. Fd. Microbiol.* **40**: 77-85

Konwar, G.C. and Joshi, D.V. 2000. Comparative effectiveness of enrichment broths for the recovery of *Salmonella* from naturally contaminated raw meats. *Indian J. Comp. Microbiol. Immunol. Inf. Dis.* **21**(1): 31-34

Kornacki, J.L. and Johnson, J.L. 2001. Enterobacteriaceae, coliforms and *Escherichia coli* as quality and safety indicators. *Compendium of Methods for the Microbiological Examination of Foods.* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington, pp.69-82

*Kwiatek, K., Wojton, B., Rola, J. and Rozanska, H. 1992. The incidence of *Listeria monocytogenes* and other *Listeria* spp. in meat, poultry and raw milk. *Bull. Vet. Inst. Pulaway* **35**: 7-11

Lancette, G.A. and Bennett, R.W. 2001. *Staphylococcus aureus* and Staphylococceal enterotoxins. *Compendium of Methods for the Microbiological Examination of Foods.* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington, pp. 387-403

Lillard, H.S. 1980. Effect on broiler carcasses and water of treating chiller water with chlorine or chlorine dioxide. *Poult. Sci.* **59**: 1761-1766

Lillard, H.S. 1990. The impact of commercial processing procedures on the bacterial contamination and cross-contamination of broiler carcasses. *J. Fd. Prot.* **53**(3): 202-204

Maxcy, R.B., Froning, G.W. and Hartung, T.E. 1973. Microbial quality of ground poultry meat. *Poult. Sci.* **52**: 486-491

*Mead, G.C., Hudson, W.R. and Hinton, M.H. 1993. Microbiological survey of five poultry processing plants in the U.K. *Br. Poult. Sci.* **34**(3): 497-503

Mercuri, A.J. and Cox, N.A. 1979. Coliforms and *Enterobacteriaceae* isolates from selected foods. *J. Fd. Prot.* **42**(9): 712-714

Miettinen, M.K., Palmu, L., Bjorkroth, K.J. and Korkeala, H. 2001. Prevalence of *Listeria monocytogenes* in broilers at the abattoir, processing plant and retail level. *J. Fd. Prot.* **64**(7): 994-999

Mikolajczyk, A. and Radkowski, M. 2002. *Salmonella* spp. on chicken carcasses in processing plants in Poland. *J. Fd. Prot.* **65**(9): 1475-1479

Mokgatla, R.M., Brozel, V.S. and Gouws, P.A. 1998. Isolation of *Salmonella* resistant to hypochlorous acid from a poultry abattoir. *Lett. Appl. Microbiol.* **27**: 379-382

Murugkar, H.V., Sherikar, A.T. and Tarwate, B.G. 1993. Studies on the microbial isolates of poultry products. *Indian J. Poult. Sci.* **28**(3): 233-237

Nair, K.K.S., Rao, D.N., Nair, R.B. and Haleem, M.A. 1990. Bacteriological quality of dressed chicken. *Indian Vet. J.* **67**: 55-58

Nkanga, E.J. and Uraih, N. 1981. Prevalence of *Staphylococcus aureus* in meat samples from traditional markets in Benin City, Nigeria and possible control by use of condiments. *J. Fd. Prot.* **44**(1): 4-8

Nordic Committee on Food Analysis. 1968b. Determination of faecal streptococci in foods (68). UDC.576.851.21

Nordic Committee on Food Analysis. 1973. Determination of number of coliform bacteria in foods. (44). UDC.576.851.48

Ojeniyi, B., Wegener, H.C., Jensen, N.E. and Bisgaard, M. 1996. *Listeria monocytogenes* in poultry and poultry products: epidemiological investigations in seven Danish abattoirs. 1996. *J. Appl. Bacteriol.* **80**: 395-401

Panda, P.C. 1971. Bacteriological condition of dressed chicken during the process of retailing. *Indian Vet. J.* **48**: 927-931

Pandurangarao, C.C. 1977. Enterotoxigenic *Staphylococci* in raw meats. *J. Fd. Sci. Technol.* **14**: 224-225

Patterson, J.T. 1972. Microbiological sampling of poultry carcasses. *J. Appl. Bacteriol.* **35**: 569-575

Pattnaik, P., Kar, B.C., Pati, P.K. and Roy, P,K. 1997. Microbial loads on chicken carcasses processed in slaughter units of Bhubaneswar City. *Indian J. Poult. Sci.* **32**(1): 89-92

Paturkar, A.M., Sherikar, A.A. and Jayarao, B.M. 1992. Prevalence of *Salmonella* in meats and sea foods of Bombay City. *J. Fd. Sci. Technol.* **29**(4): 242-243

*Peresi, J.T.M., Almeida, I.A.Z.C.D., Lima, S.I., Fernandes, S.A., Tavechio, A.T. and Gelli, D.S. 1999. *Salmonella*: serotyping and antimicrobial susceptibility patterns of strains isolated from chicken carcass samples

commercialized in the Sao Jose do Rio Preto area sp. *Revista do Instituto Adolfo Lutz* **58**(1): 41-46

Pini, P.N. and Gilbert, R.J. 1988. The occurrence in the U.K. of *Listeria* species in raw chickens and soft cheeses. *Int. J. Fd. Microbiol.* **6**: 317-326

Plummer, R.A.S., Blissett, S.J. and Dodd, C.E.R. 1995. *Salmonella* contamination of retail chicken products sold in the UK. *J. Fd. Prot.* **58**(8): 843-846

Rahkio, T.M. and Korkeala, H.J. 1997. Airborne bacteria and carcass contamination in slaughter houses. *J. Fd. Prot.* **60**(1): 38-42

Raj, B.R. 2002. Bacteria of public health significance in broiler dressed chicken. M.V.Sc. thesis, Kerala Agricultural University, Trichur, p. 92

Rajvaidya, N. and Markandey, D. 1992. *Advances in Environmental Science and Technology, Water Characteristics and Properties.* APH Publishing Corporation, New Delhi, p. 372

Rangaswamy, R. 1995. *A Texbook of Agricultural Statistics.* New Age International Publishers Ltd., New Delhi. p. 495

Rao, D.N. and Ramesh, B.S. 1992. The microbiology of sheep carcasses processed in a modern Indian abattoir. *Meat Sci.* **32**: 425-536

Rao, D.N., Nair, K.K.S. and Sakhare, P.Z. 1998. Meat microbiology and spoilage in tropical countries. *The Microbiology of Meat and Poultry.* (eds. Davies, A. and Board, R.) Blackie Academic and Professional, London, pp. 220-265

Samadpour, M., Ongerth, J.E., Liston, J., Tran, N., Nguyen, D., Whittam, T.S., Wilson, R.A. and Tarr, P.I. 1994. Occurrence of shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork and

poultry from grocery stores in Seattle, Washington. *Appl. Environ. Microbiol.* **60**(5): 1038-1040

Sarlin, L.L., Barnhart, E.T., Caldwell, D.J., Moore, R.W., Byrd, J.A., Caldwell, D.Y., Corrier, D.E., Deloach, J.R. and Hargis, B.M. 1998. Evaluation of alternative sampling methods for *Salmonella* critical control point determination at broiler processing. *Poult. Sci.* 77(8): 1253-1257

Sauter, E.A., Petersen, C.F. and Parkinson, J.F. 1978. Microfloral comparison of fresh and thawed frozen fryers. *Poult. Sci.* **57**: 422-424

Seneviratna, P., Robertson, J., Robertson, I.D. and Hampson, D.J. 1990. *Listeria* species in foods of animal origin. *Aust. Vet. J.* **67**(10): 384

Sharma, D.K., Singh, N. and Joshi, D.V. 1995. *Escherichia coli* in milk, meat and meat products: isolation, characterization, antibiogram and zoonotic significance. *J. Fd. Sci. Technol.* **32**(5): 409-412

Sharma, V.D., Dixit, V.P. and Joshi, R.K. 1995. Occurrence of *Salmonella* serovars in foods of animal origin with special reference to antibiogram and enterotoxigenicity. *J. Fd. Sci. Technol.* **32**(3): 221-223

Simmons, M., Fletcher, D.L., Cason, J.A. and Berrang, M.E. 2003. Recovery of *Salmonella* from retail broilers by a whole-carcass enrichment procedure *J. Fd. Prot.* **66**(3): 446-450

Smith, H.R. and Cheasty, T. 1998. Diarrhoeal disease due to *Escherichia coli* and *Aeromonas*. *Topley and Wilson's Microbiology* and *Microbial Infections. II. Systematic Bacteriology* (eds. Balows, A. and Duerden, B.I.). Oxford University Press, New York, pp. 513-529

Soriano, J.M., Rico, H., Molto, J.C. and Manes, J. 2001a. Incidence of microbial flora in lettuce, meat and Spanish potato omelette from restaurants. *Fd. Microbiol.* **18**: 159-163

Soriano, J.M., Rico, H., Molto, J.C. and Manes, J. 2001b. *Listeria* species in raw and ready-to-eat foods from restaurants. *J. Fd. Prot.* **64**(4): 551-553

Swanson, K.M.J., Petran, R.L. and Hanlin, J.H. 2001. Culture methods for enumeration of microorganisms. *Compendium of Methods for the Microbiological Examination of Foods.* (eds. Downes, F.P. and Ito, K.). Fourth edition. American Public Health Association, Washington, pp. 53-67

Tarwate, B.G., Sherikar, A.T. and Murugkar, H.V. 1993. Microbiological analysis of environmental sources of contamination in Deonar abattoir. *J. Fd. Sci. Technol.* **32**(2): 127-129

Telo, A., Beli, E., Dibra, A. and Panariti, E. 1998. *Salmonella enteritidis* in imported poultry meat in Albania. *Vet. Arhiv.* **68**(5): 173-176

The Food Act Government of Mauritius. 1998. Microbiological standards for specified food. Eighth schedule. Regulation. 62(2)b.

*Turtura, G.C. 1991. *Enterobacteriaceae* and other gram negative bacteria in slaughtered poultry. *Microbiologie-Aliments-Nutrition* **9**(2): 139-146

Uyttendaele, M., Troy, P.D. and Debevere, J. 1999. Incidence of *Salmonella, Campylobacter jejuni, Campylobacter coli* and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. *J. Fd. Prot.* **62**(7): 735-740

Varabioff, Y. 1990. Incidence and recovery of *Listeria* from chicken with a pre-enrichment technique. *J. Fd. Prot.* **53**(7): 555-557

Vorster, S.M., Greebe, R.P. and Nortje, G.L. 1994. Incidence of *Staphylococcus aureus* and *Escherichia coli* in ground beef, broilers and processed meats in Pretoria, South Africa. *J. Fd. Prot.* **57**(4): 305-310

172253

Wang, G., Yan, K., Feng, X., Chen, S., Lul, A. and Kokubo, Y. 1992. Isolation and identification of *Listeria monocytogenes* from retail meats in Beijing. *J. Fd. Prot.* **55**(1): 56-58

Whittemore, A.D. 1993. A modified most probable number technique to enumerate total aerobes, enterobacteriaceae and *Salmonella* on poultry carcasses after the whole carcass rinse procedure. *Poult. Sci.* **72**: 2353-2357

Wong, H., Chao, W. and Lee, S. 1990. Incidence and characterization of *Listeria monocytogenes* in foods available in Taiwan. *Appl. Environ. Microbiol.* **56**(10): 3101-3104

Yagoub, I.A. and Mohamed, T.E. 1987. Isolation and identification of *Salmonella* from chickens in Khartoum province of the Sudan. *Br. Vet. J.* **143**(6): 537-540

*Yashoda, K.P., Sachindra, N.M., Sakhare, P.Z. and Narasimharao, D. 2001. Microbiological quality of broiler chicken carcasses processed hygienically in a small scale poultry processing unit. *J. Fd. Quality* **24**(3): 249-259

Zivkovic, J., Jaksic, S. and Miokovic, B. 1997. *Salmonella* serovars in chicken meat and chicken meat products in Zagreb, Croatia. *Vet. Arhiv.* **67**(4): 169-175

* Originals not consulted

EVALUATION OF BACTERIOLOGICAL QUALITY OF PROCESSED CHICKEN

RAJI ROSE JACOB

Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

2003

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

ABSTRACT

An investigation was carried out to evaluate the bacterial quality of chicken carcasses collected at two steps on the dressing line of a meat processing plant. During the study a total of 60 dressed chicken carcasses consisting of 30 each were randomly collected after the removal of head and feet (ARHF) and after evisceration (AE) from a meat processing plant located at Cochin. The bacterial quality was evaluated by estimating the total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC). All the samples were examined for the presence of *Escherichia coli*, salmonellae, *Staphylococcus aureus* and *Listeria monocytogenes.* Bacterial quality of environmental samples and processing equipments were also evaluated.

The study revealed a significant ($P<0.05$) difference between the mean total viable count of the samples taken ARHF and AE. A higher mean count ($5.88 \pm 0.13\ \log_{10}$ cfu/ml) was observed in the former group of carcasses. The count of the carcasses taken after the removal of head and feet ranged between 10^4 and 10^7 cfu/ml while that of eviscerated carcasses vary from 10^4 to 10^6 cfu/ml.

A higher mean coliforms count ($3.92 \pm 0.12\ \log_{10}$ cfu/ml) was observed in the samples taken from the carcasses after evisceration. The count of the carcasses in both groups varied between 10^2 and 10^5 cfu/ml.

Analysis of variance test of the mean *Escherichia coli* count revealed significant ($P<0.05$) difference between the count of the samples taken from the carcasses ARHF and AE. The eviscerated carcasses had higher count ($2.15 \pm 0.24\ \log_{10}$ cfu/ml). The mean *Escherichia coli* count of the carcasses taken ARHF was $0.89 \pm 0.23\ \log_{10}$ cfu/ml. In both groups, the counts ranged from 10^2 to 10^3 cfu/ml. *Escherichia coli* was not detected in 63.33 per cent of carcasses taken after the removal of head and feet and 23.33 per cent of eviscerated carcasses.

All samples collected from the carcasses after the removal of head and feet and after evisceration had faecal streptococci but the mean count of the samples

belong to both groups did not differ significantly. A higher mean count (3.91 ± 0.07 log_{10} cfu/ml) was obtained in the samples collected from the eviscerated carcasses. The samples taken after the removal of head and feet had a mean count of 3.89 ± 0.06 log_{10} cfu/ml.

Correlation coefficient of the data revealed a significant ($P<0.05$) and positive association between the mean TVC and FSC, CC and ECC, CC and FSC and ECC and FSC of the samples taken from the carcasses after the removal of head and feet. A similar relationship was observed between the mean CC and ECC and ECC and FSC of the eviscerated carcasses.

Escherichia coli was isolated from 36.67 per cent of carcasses taken after the removal of head and feet and 76.67 per cent of eviscerated carcasses. A total of 40 *Escherichia coli* isolated from both the groups were serotyped and grouped under 15 serotypes, two rough strains and one untypable. The serotypes consisted of O2, O5, O8, O16, O21, O51, O60, O64, O69, O78, O81, O101, O119, O123 and O140.

Salmonella enteritidis was isolated from three (10 per cent) carcasses taken after the removal of head and feet.

Eleven (36.67 per cent) samples taken from the carcasses after evisceration revealed the presence of *Staphylococcus aureus.*

Listeria monocytogenes could not be isolated from both the groups of carcasses.

The mean total viable count of air samples from the processing hall was high (1.99 ± 0.83 log_{10} cfu/min) followed by sticking and bleeding area (1.63 ± 0.98 log_{10} cfu/min) and scalding area (1.54 ± 0.01 log_{10} cfu/min). The water samples from the scalding tank had TVC, CC, ECC and FSC but pond and tap water samples, defeathered carcass wash and final carcass wash did not reveal the presence of *Escherichia coli* and faecal streptococci. Among the equipments, samples from the eviscerating table had TVC, CC and FSC but the samples from the defeathering machine and knife swab had only TVC and CC.