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**EVALUATION OF BACTERIOLOGICAL  
QUALITY OF PORK CARCASSES IN  
A PROCESSING PLANT**

**SHINY JOHN**

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

**Master of Veterinary Science**

**Faculty of Veterinary and Animal Sciences  
Kerala Agricultural University, Thrissur**

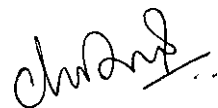
**2003**

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

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

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**CERTIFICATE**

Certified that this thesis entitled “EVALUATION OF BACTERIOLOGICAL QUALITY OF PORK CARCASSES IN A PROCESSING PLANT” is a record of research work done independently by Dr. SHINY JOHN under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

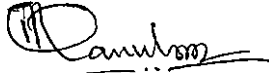
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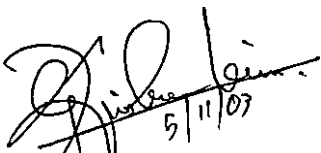


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## ACKNOWLEDGEMENT

*I will instruct you and guide you along the best pathway for your life, I will advise you and watch your progress*      *Ps 32:8*

Fervidly and obsequiously, may I place on record my sincere and heart felt gratitude to Major Advisor and Chairman of my advisory Committee, **Dr. E. Nanu**, Professor and Head, Department of Veterinary Public Health and Dean (i/c), College of Veterinary and Animal Sciences, Mannuthy for his meticulous guidance, personal attention, keen interest, ineffable cuddles and cossets, incessant support and encouragement, paternal affection and timely advice during the entire study period, inspite of his hectic schedule. His unruffled, pragmatic and easy-going disposition has helped me a lot in correcting many of my naïve goofs, as a student. I reckon it a privilege and matter of pride to work under the potential guidance of a versatile academician of his caliber and acumen.

I extend my sincere thanks to **Dr. C. Latha**, Assistant Professor, Department of Veterinary Public Health and member of my Advisory Committee for her timely advice, understanding, concrete suggestions, affable manners and generosity in sharing her scholastic eminence and expertise related to the topic of research.

It is my privilege to accentuate the most sincere sense of gratitude to **Dr. George T. Oommen**, Associate Professor and Director, Centre of Excellence in Meat Science and Technology, and member of my Advisory Committee for his valuable help, encouraging support and large, wide spread comprehensive constructive suggestions, during the course of study. Despite, the preoccupation of holding charge of many offices at a time, he spared much of his precious time in evaluating the thesis. His indefatigable sense of dedication to duty, personal discipline, hard work, innovative approach towards continual improvement, uncanny ability to analyse, plan and arrange whatever he sets to do and the 'never-say-die' commitment to do the very best in all the endeavours have always enthused me, as a scholar.

I owe a special word of thanks to **Dr. G. Krishnan Nair**, Associate Professor, Department of Microbiology and member of my Advisory Committee, for all the help and guidance.

I gratefully cherish the acquaintance with **Dr. B. Sunil**, Assistant Professor, Department of Veterinary Public Health. Besides, being an efficient preceptor, he used to fathom and resolve many of my research-bound puerile, querulous dubiousness through pep talks and rekindle to work, with a subtle, but genial, supportive, fraternal heed and concern. His sincere welcoming of learner's discernment and ability has helped me in amassing the spirit of knowledge in the various facets of the discipline and bringing out the research to fruitful completion.

With exquisite pleasure, I am extremely obliged to **Dr. Ani. S. Das**, Managing Director, MPI, Koothattukulam, **Dr. Saji**, **Dr. Sathu** and **Mr. James Mathew** for permitting me to carry out my research work successfully at MPI. This endeavour turned out to be a dream-come-true solely by the whole hearted and unstained love, concern, co-operation and help rendered by all the processing line workers at MPI.

*Within the limits of lexicon, I owe my utmost and sincere feelings of gratitude to all of them.*

*My sincere gratitudes are due to Dr. P. Kuttinarayanan, Associate Professor and Head, Centre of Excellence in Meat Science and Technology, for permitting me to utilise the facilities at the department.*

*I express my deep felt thanks to my teachers, Dr. Mini, Dr. K. Anilkumar, Dr. Sisilamma George, Dr. A.M. Chandrasekharan, Dr. P.R. Chandrasekharan, and Dr. P.I. Geevarghese for their whole-hearted help in different forms during the study.*

*I am in short of words to express my most sincere gratitude to Dr. Colin.O. Gill (Canada) and Dr. Tiina Autio (Finland), who, from miles across, were kind enough to send me their research articles on time.*

*I am indeed overwhelmed and grateful to Dr. H.G. Brahmne, Joint Director and Head, National Salmonella and Escherichia Centre, Central Research Institute, Kasauli for sending me the needful information on Escherichia coli O157 and also for serotyping the Escherichia coli isolates and sending results on time.*

*I am grateful to Smt. M. Sujatha, Department of Statistics for the help rendered in statistical analysis.*

*My sincere gratitudes are due to Dr. Deepa Jolly, Dr. Vrinda, Dr. Binsy, Dr. Anish, Dr. Bindu and Dr. Ambily for their help and co-operation.*

*The services of non teaching staffs of Department of Veterinary Public Health, Miss. Sandhya, Mrs. Deepa, Mr. Sundaran and Mrs. Thara are deeply acknowledged.*

*I am thankful to the Dean (i/c), College of Veterinary and Animal Sciences, Mannuthy and the Associate Dean, College of Veterinary and Animal Sciences, Pookot for providing all the necessary facilities for research work.*

*I am greatly obliged to the help and services rendered by Mrs. Shylaja and Mr. Radhakrishnan at the library of Central Institute of Fisheries Technology, Kochi.*

*The help extended by librarian and other staff of College library, Central library (KAU) and ARIS cell are also deeply acknowledged.*

*I express my sincere gratitude to the Kerala Agricultural University for providing me the fellowship for the post graduate programme.*

*I also place a word of thanks to Mr. Thomas for providing the transportation facilities to Koothattukulam.*

*I take great pleasure in expressing my heart-felt gratitude to Babuchayan, Saji Uncle, Shirley Auntie, Savin and Shilpa for their love, concern and mental support during the study.*

*I am extremely grateful for the bonhomie hospitality extended to me by Shantha Auntie, Rekha and Arun. I am really obliged to them for making me 'feel at home', whenever I went to their home for thesis correction.*

*I would like to record special thanks for the cordial friendship and exhilarating moments, shared by my dear colleagues Dr. Raji and Dr. Sethulekshmi, that really enabled a strenuous task smooth sailing.*

*The timely help, support and friendship extended by Dr. PX Antony, Dr. Kantharaj, Dr. Sai Prasad, Dr. Suresh, Dr. Sasi, Dr. Sekhar, Dr. Sakthivel, Dr. Sethu C. Nair, Dr. Deepa Surendran, Dr. Mrudula, Dr. Shibi, Dr. Uma and Dr. Geetha are deeply acknowledged.*

*The invaluable love support and help in varied forms by my bonvivant Dr. Neena Simon, Dr. Thushara, Dr. Mejo, Dr. Lydia, Dr. Leeba and Dr. Deepa Gopinath are deeply remembered from the depths of my heart.*

*I express my sincere thanks to my sisterly friend, Dr. Durgga, who always walks an extra-mile to brighten up my days through her mental support, affectionate friendship, timely advices and sustained encouragement, which instilled fortitude to overcome the hardships faced during the period of study and research work.*

*I am extremely obliged to Mr. Sachi and Mr. Deepak (Ms/Josco) for the processing of photograph.*

*A word of special thanks to Mrs. Rema Nair and Mrs. Simi for their skill, unflagging patience, precision and promptness in the preparation of this manuscript.*

*Thanks are due to Rao Sir, Lalitha Auntie, Vidya and Mayuri for their support, blessings and encouragement.*

*I thank all the members of VCPF for their support through prayers.*

*I am also thankful for the support, love and care shown by all at the Department of Livestock Products Technology.*

*I thankfully remember all those, who directly or indirectly helped me and contributed to finalise the work*

*Words are incapable to express my feeling to my Pappa, Mummy and Shibu for their prayers, blessings and incessant encouragement which helped me to complete the course successfully.*

*Above all, I kneel before the Almighty for abiding in His promises and showering me all the undeserved blessings and grace without which the successful completion of the course would not have materialised.*

**Shiny John**

**DEDICATED**  
**TO**  
***THE HOLY TRINITY***



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# Introduction

## 1. INTRODUCTION

Meat is one of the most important livestock product and is also highly perishable, but play a significant role in human nutrition. The production of meat in India in 1980 was 0.85 million metric tonnes. In 1990 and 2000, the meat production of the country was 3.8 and 4.6 million metric tonnes. This indicates that the meat production in the country showed a progressive rise during the past two decades. The per capita consumption of meat in the country is only 9.5 g/day with is far below from 30 g/day recommended by ICMR. The meat production in the country primarily depends on the livestock. India has the highest livestock population in the world and the present meat production scenario is 219.6 million cattle, 94.1 million buffaloes, 58.2 million sheep, 123.5 million goat, 17.5 million pigs and 413 million poultry. In spite of many restrictions, pig is the only species reared exclusively for meat production. Due to its high prolificacy, efficient feed conversion into edible meat, short generation interval, fast growth rate, low cost of price of pork, larger availability of meat in terms of quantity and energy than other livestock species and other biological advantages, pigs play an important role to make up the increasing protein demand. In 2000, world pork production was 8.9 million tonnes of which India contributed 560 thousand tonnes. In Kerala, the pork production depends on 1.43 lakh pigs, which is one per cent of total pig population in the country. Despite, religious taboos there is an increased demand for pork and its products in the state.

In India, meat is produced in 3600 abattoirs which are under the control of local authorities. The infrastructure facilities and hygienic conditions prevailing in majority of the slaughterhouses are far below from satisfactory. In 1970's, eight bacon factories were established in different states of the country for the production and processing of pork. Most of these bacon factories have now been closed. At present, 24 processing plants are operating under private sector for the production and processing of meat to meet the export trade in the country.

During the production of pork, pigs pass through various operations viz., antemortem inspection, stunning, sticking, scalding, dehairing, singeing, evisceration, washing and chilling or processing. During these process, pig is being

handled by many workers and are being exposed to different equipments and also to the environment. All these activities lead to bacterial contamination of pork. The contaminants include spoilage or saprophytic organisms and also pathogenic organisms. Contamination of pork with the former group of organisms and their subsequent growth and multiplication on the carcasses leads to spoilage of meat which results in economic loss to the meat trade and the loss of valuable animal product. However, the growth and multiplication of the latter group of organisms may not produce appreciable change on the carcass or meat but produce foodborne infection and intoxication in the consumers. On perusal of literature, it is observed that the data on the bacteriological quality of pork particularly on carcasses in the dressing line is scanty and hence the present study was undertaken with the following objectives:

1. To assess bacterial quality of pork carcasses collected from two sites of the dressing line by estimating
  - a. Total viable count
  - b. Coliforms count
  - c. *Escherichia coli* count
  - d. Faecal streptococcal count
2. To detect the presence of bacteria of public health significance viz.,
  - a. *Escherichia coli*
  - b. *Staphylococcus aureus*
  - c. *Salmonella*
  - d. *Listeria monocytogenes*
3. To evaluate the role of environment, processing equipments and personnel involved in the dressing line, in the contamination of the carcasses by estimating the bacterial load, as indicated in the first objective.

# *Review of literature*

## 2. REVIEW OF LITERATURE

### 2.1 BACTERIAL COUNT

#### 2.1.1 Total viable count

Salm *et al.* (1978) evaluated the microbial load of three groups of skinned hog carcasses. The mean total plate count at ham, belly and shoulder of skinned carcasses were  $254 \pm 86$ ,  $153 \pm 47$  and  $522 \pm 117$  organisms/cm<sup>2</sup>. The corresponding count for scalded carcasses were  $969 \pm 162$ ,  $140 \pm 28$  and  $102 \pm 28$  organisms/cm<sup>2</sup>.

The total aerobic plate count of the samples from native pig carcasses varied from  $1.1 \times 10^5$  to  $1.07 \times 10^6$  per gram (Nair *et al.*, 1984).

The mean total viable count for excision and swab samples from an abattoir where carcasses were scalded by tank scalding were  $4.036 \log_{10}$  count/cm<sup>2</sup> and  $3.712 \log_{10}$  count/cm<sup>2</sup>, respectively. The corresponding count for samples from another abattoir where steam chamber was used for scalding were  $4.074 \log_{10}$  count/cm<sup>2</sup> and  $3.735 \log_{10}$  count/cm<sup>2</sup>, respectively (Morgan *et al.*, 1985).

Scriven and Singh (1986) reported that the mean total plate count of pork rump was  $4.1 \times 10^6$ /g and for minced pork samples was  $5.1 \times 10^7$ /g.

Gupta *et al.* (1987) assessed the bacteriological quality of fresh pork samples collected from modern, semi-modern and rural slaughterhouses and two retail shops at 15 days interval. The mean standard plate count of samples belonging to modern, semi modern and rural slaughterhouses was 8.08, 5.38 and 5.77  $\log_{10}$ /g, respectively. The count in the samples belonging to one of the shop was 9.14  $\log_{10}$ /g and that from the other shop was 8.11  $\log_{10}$ /g.

The mean total viable count per gram of pork samples obtained from meat laboratory was  $0.08 \times 10^6$  and that from local market was  $44.3 \times 10^6$  (Borah *et al.*, 1992).



Rahkio and Korkeala (1992) evaluated the microbial contamination level of pork carcasses during the slaughtering process. The mean total aerobic count of unbrushed and brushed eviscerated carcasses was  $3.29 \pm 0.41$  and  $3.32 \pm 0.46 \log_{10}/\text{cm}^2$ , respectively. The ham region of unbrushed and brushed carcasses revealed a count of  $3.31 \pm 0.36$  and  $3.43 \pm 0.42 \log_{10}/\text{cm}^2$ .

Gill and Bryant (1993) sampled pig carcasses, leaving dehairing machine and polishing machine on six days, at two plants A and B. Carcasses leaving the dehairing equipment at plant A had total numbers between  $9.73 \times 10^3$  and  $1.0 \times 10^4$  cfu/cm<sup>2</sup> and those at plant B had the count between  $1.2 \times 10^4$  and  $7.9 \times 10^4$  cfu/cm<sup>2</sup>. Carcasses leaving the polisher at plant A yielded total count in the range of  $1.2 \times 10^3$  and  $2.2 \times 10^3$  cfu/cm<sup>2</sup> and the corresponding count of samples taken from plant B was in the range of  $2.8 \times 10^4$  to  $5.5 \times 10^4$  cfu/cm<sup>2</sup>.

Gill *et al.* (1995) determined the total number of bacteria in a batch of five polished uneviscerated carcasses selected randomly. The mean count on back, waist, belly and foreleg was  $7.9 \times 10^2$ ,  $4.7 \times 10^2$ ,  $8 \times 10^2$  and  $2.0 \times 10^3$  per cm<sup>2</sup>, respectively.

Sharpe *et al.* (1996) obtained total viable count ranging from 3.54 to 4.02  $\log_{10}$  cfu/cm<sup>2</sup> on skin of neck areas of pork carcasses.

Gill and Jones (1997a) assessed the hygienic characteristics of a dressing process of pasteurised pig carcasses. The mean total aerobic count of the carcasses entering the dressing process was  $0.57 \log_{10}/\text{cm}^2$  and the count on the carcasses that exit the process was  $2.59 \log_{10}/\text{cm}^2$ .

Gill *et al.* (1997) evaluated the bacterial quality of pig carcasses in two sets, selected randomly from those entering or leaving pasteurisation treatment during normal commercial operation. The estimated mean bacterial count in the samples belonging to set one was  $2.38 \log_{10}/\text{cm}^2$  and in the other set, the count was  $2.25 \log_{10}/\text{cm}^2$ .

The mean aerobic count of the samples obtained from head, diaphragm, belly-sternum and pelvic cavity of pork carcasses was 5.32, 4.63, 5.65 and 5.28 log cfu/g, respectively (Miller *et al.*, 1997).

Rahkio and Korkeala (1997) evaluated the level of airborne bacteria on 58 pork carcasses collected from four slaughter houses, A, B, C and D. The mean bacterial count at the neck and abdomen area on pig carcasses in slaughterhouses B and D was  $2.55 \pm 0.69$  log cfu/cm<sup>2</sup> and  $2.81 \pm 0.65$  log cfu/cm<sup>2</sup> respectively. The corresponding count of the samples collected from slaughterhouses, A and C was  $3.76 \pm 0.49$  log cfu/cm<sup>2</sup> and  $3.34 \pm 0.39$  log cfu/cm<sup>2</sup>.

Carr *et al.* (1998) evaluated the chilling and trimming effects on the microbial populations of 30 pork carcasses. The mean aerobic plate count of hot fat trimmed and not fat trimmed carcasses was  $5.9 \pm 5.4$  and  $5.5 \pm 5.4$  log<sub>10</sub> cfu/cm<sup>2</sup>, respectively.

Gill and Jones (1998) evaluated the bacterial quality of two sets of pig carcasses, at neck site, subjected to three operations in a modified pig carcass dressing process comprising of carcass polishing, cutting open the throat and floor of mouth and after pasteurisation. In the first process, carcasses belonging to set one had a mean total aerobic count of 1.90 log cfu/cm<sup>2</sup>. The count of samples taken after cutting open the throat and floor of mouth and after pasteurisation was 2.95 and 0.98 log cfu/cm<sup>2</sup>. The second set of carcasses, yielded aerobic count of 1.93, 3.06 and 1.26 log cfu/cm<sup>2</sup> at the three stages, respectively.

Gill *et al.* (1998) evaluated the effect of hot water pasteurising treatment on bacterial load of pig carcasses. The mean total aerobic count on the carcasses before treatment of unsplit carcasses was 3.22 log cfu/cm<sup>2</sup> and the corresponding count on split carcasses was 2.81 log cfu/cm<sup>2</sup>.

Waskar *et al.* (1998) reported that the mean total mesophilic aerobic plate count of deboned and minced pork samples as 6.483 and 7.258 log cfu/g, respectively.

Palumbo *et al.* (1999) compared the efficiency of excision and swabbing methods to determine the microbial quality of pork carcass surface. The study revealed that the mean aerobic plate count by one site swab method at belly was  $3.29 \pm 0.52 \log \text{cfu/cm}^2$  and the count by three-site swab method at jowl, belly and ham was  $4.15 \pm 0.64 \log \text{cfu/cm}^2$ .

Yu *et al.* (1999) evaluated the bacterial quality of pork carcasses selected randomly from the various stages of processing. The study revealed that the samples collected from dehaired carcasses had mean aerobic plate count of 3.31, 1.83, 1.88 and 1.03  $\log_{10} \text{cfu/cm}^2$  before first polishing, after second singeing, before chilling and after chilling, respectively.

Gill and Jones (2000) compared the efficiency of recovery of bacteria by excision and swabbing with cellulose acetate sponge, medical gauze and cotton wool. Samples were collected from two groups of pig carcasses leaving spray cooling process of a pork packing plant. The mean total aerobic count recovered from carcasses belonging to group one was 2.78, 2.24, 2.32 and 2.29  $\log_{10}/\text{cm}^2$  in samples collected by excision, sponge, gauze and cotton wool swabbing, respectively. The corresponding count of the samples obtained by these methods in the group two carcasses were 2.37, 2.35, 2.38 and 2.12  $\log_{10}/\text{cm}^2$ .

Gill *et al.* (2000a) evaluated the microbiological quality of pig carcasses at eight packing plants, viz., A, B, C, D, E, F, G and H. The mean total aerobic count of samples collected from these plants after dressing were 3.78, 3.47, 3.25, 3.04, 2.54, 2.33, 2.54 and 1.83  $\log_{10} \text{cfu/cm}^2$ , respectively.

Gill *et al.* (2000b) examined the count of two sets of pig carcasses leaving the dressing process. The mean aerobic count obtained in each sets was 3.16  $\log_{10} \text{cfu/cm}^2$  and 3.47  $\log_{10} \text{cfu/cm}^2$ , respectively.

Rivas *et al.* (2000) estimated the microbial contamination of pig carcasses at post bleeding, post scalding, post dehairing, post scraping and at the end of line. The study revealed that the highest mean aerobic plate count in samples collected at post bleeding was  $4.68 \pm 0.15 \log \text{cfu/cm}^2$  and the lowest count ( $2.54 \pm 0.56$ )  $\log$

cfu/cm<sup>2</sup> in samples collected at post scalding. The mean count of samples taken at post dehairing, post scraping, post evisceration and at end of line was  $4.26 \pm 0.31$ ,  $3.72 \pm 0.32$ ,  $3.53 \pm 0.22$  and  $3.81 \pm 0.28$  log cfu/cm<sup>2</sup>, respectively. During the investigation the bacterial quality of unwashed non-GMP carcasses, unwashed, GMP carcasses and washed GMP carcasses were also estimated. The mean aerobic plate count of unwashed non GMP carcasses was  $3.91 \pm 0.26$  log cfu/cm<sup>2</sup>. The mean count of the unwashed, GMP carcasses and washed GMP carcasses was  $3.96 \pm 0.32$  and  $3.66 \pm 0.50$  log cfu/cm<sup>2</sup>, respectively.

Castelo *et al.* (2001a) evaluated the microbial load on lean and fat trim pork loins obtained from abattoir and reported that the lean pork trim had a mean aerobic population of  $4.82 \pm 0.32$  log<sub>10</sub> cfu/cm<sup>2</sup>.

Gill and Badoni (2001) evaluated the microbiological effects of a pasteurisation treatment and subsequent trimming of sticking wounds, in pig carcasses. The mean total aerobic count of carcass samples collected before pasteurisation was  $2.18$  log<sub>10</sub>/cm<sup>2</sup>. The samples taken after pasteurisation had a mean count of  $1.97$  log<sub>10</sub> cfu/cm<sup>2</sup>, whereas, the samples collected after trimming the sticking wound had a mean count of  $2.26$  log<sub>10</sub> cfu/cm<sup>2</sup>.

Gill *et al.* (2001) evaluated the recovery rates of bacteria by excision and swabbing with cotton wool, cellulose acetate sponge and gauze from sets of fat and lean pork loins. The mean aerobic plate count of samples collected from fat loins by excision, swabbing with cotton wool, cellulose acetate sponge and gauze was  $3.51$ ,  $2.89$ ,  $2.96$  and  $3.12$  log<sub>10</sub>/cm<sup>2</sup>, respectively. The corresponding count of samples collected from lean loins was  $3.37$ ,  $3.01$ ,  $2.78$  and  $2.31$  log<sub>10</sub>/cm<sup>2</sup>.

Hansson (2001) determined the microbiological quality of pork carcasses, each from four high capacity slaughterhouses and four low capacity slaughter houses. The mean aerobic microorganisms on the pork carcasses was  $3.44$  log<sub>10</sub> cfu/cm<sup>2</sup> at high capacity slaughter houses and  $3.34$  log<sub>10</sub> cfu/cm<sup>2</sup> at low capacity slaughter houses.

Yu *et al.* (2001) determined the efficiency of excision and sponge swabbing methods in the recovery of bacteria from jowl area of prechill and post chill pig carcasses. The mean aerobic count of prechilled carcasses by excision method was  $2.54 \log \text{ cfu/cm}^2$  and by swabbing the count was  $1.61 \log \text{ cfu/cm}^2$ . The count by excision and swabbing of post chill carcasses were 1.69 and  $1.07 \log \text{ cfu/cm}^2$  respectively.

Bolton *et al.* (2002) examined pigs to evaluate the number of bacteria on live animals before transport to abattoir and on carcasses after power hosing, bleeding, scalding-dehairing, singeing, preevisceration power-hosing, evisceration, final washing and chilling. The carcasses after final washing had total aerobic count that ranged from 3.6 to  $4.0 \log_{10} \text{ cfu/cm}^2$ .

### 2.1.2 Coliforms count

Nair *et al.* (1984) determined the bacteriological quality of native pig carcasses by MPN method and reported that the coliforms count of the samples ranged from  $2.5 \times 10^1$  to  $3.2 \times 10^3/\text{g}$ .

Scriven and Singh (1986) assessed the microbial quality of retail pork rump and minced pork samples. The mean MPN of coliforms of the former samples was 119 cells /g and that of the latter samples was 2091 cells/g.

Gupta *et al.* (1987) evaluated the influence of abattoir types and retail shops on the bacterial quality of pork. During the investigation, samples were collected from two retail shops and from modern, semi-modern and rural abattoir types. The mean MPN coliforms of the samples obtained from modern and semi-modern abattoirs was 6.85 and  $4.30 \log_{10}/\text{g}$ , respectively. The MPA of the samples from rural abattoir was  $4.5 \log_{10}/\text{g}$ . The mean MPN of coliforms in the samples belonging to one of the retail shops was  $7.81 \log_{10}/\text{g}$  and the count of samples from the other retail shop was  $6.44 \log_{10}/\text{g}$ .

Borah *et al.* (1992) assessed the bacteriological quality of pork samples collected from meats laboratory and from local market and reported that 47.37 per cent of the isolates were coliforms.

Gill and Jones (1997a) assessed the hygienic characteristics of a dressing process of pasteurised pig carcasses. The hygienic effect of selected operations was examined by sampling an appropriate site on carcasses entering and leaving the operation. None of the carcasses entering the process had coliforms but carcasses that exit the process had mean coliforms count of  $1.77 \log_{10} \text{ cfu}/100 \text{ cm}^2$ .

Gill *et al.* (1997) evaluated the bacterial quality of pig carcasses in sets of two, selected randomly from those entering or leaving pasteurisation treatment. The estimated mean coliforms count in the samples belonging to set one was  $4.31 \log_{10}/100 \text{ cm}^2$  and in the other set, the count was  $3.65 \log_{10}/100 \text{ cm}^2$ .

Carr *et al.* (1998) assessed the effect of chilling and trimming on the microbial populations of pig carcasses. The mean coliforms count of hot fat trimmed normal and freeze chilled carcasses was 2.4 and  $2.7 \log_{10} \text{ cfu}/\text{cm}^2$ , respectively. The non-fat trimmed normally chilled carcasses had a mean coliforms count of  $3.9 \log_{10} \text{ cfu}/\text{cm}^2$  and the count in the non-fat trimmed freeze chilled carcasses was  $3.0 \log_{10} \text{ cfu}/\text{cm}^2$ .

Gill and Jones (1998b) examined the bacterial quality of two sets of pig carcasses. The samples from each carcass was selected from neck site after polishing, cutting open the throat and floor of mouth and pasteurisation. The mean coliforms count of carcasses in set one after polishing was  $0.71 \log_{10} \text{ cfu}/100 \text{ cm}^2$  and the count in the samples collected after cutting open the throat and floor of mouth was  $2.82 \log_{10} \text{ cfu}/100 \text{ cm}^2$ . However, the total number of organisms on pasteurised carcasses was  $1.20 \log_{10} \text{ cfu}/2500 \text{ cm}^2$ . For the second set of carcasses, the mean coliforms count of samples collected after cutting open the throat and floor of mouth was  $3.02 \log_{10} \text{ cfu}/100 \text{ cm}^2$ .

Gill *et al.* (1998) assessed the effect of hot water pasteurisation on the bacterial quality of pig carcasses selected randomly. The mean coliforms count on

unsplit carcasses prior to treatment was  $1.78 \log_{10} \text{ cfu}/100 \text{ cm}^2$ . However, total number of coliforms recovered from treated unsplit carcasses was  $0.48 \log/2500 \text{ cm}^2$ . The total coliforms recovered from pasteurized and unpasteurised split carcasses were  $3.39 \log \text{ cfu}/2500 \text{ cm}^2$  and  $0.60 \log \text{ cfu}/2500 \text{ cm}^2$ , respectively.

Laubach *et al.* (1998) evaluated the microbiological quality of hot-boned head meat samples and reported that the coliforms level of samples was  $2.37 \pm 0.42 \log_{10} \text{ cfu/g}$ .

Waskar *et al.* (1998) evaluated the microbiological profile of raw, semi processed pork products and ingredients used in manufacture of processed pork products. The study revealed that deboned meat had a mean coliforms count of  $4.243 \log \text{ cfu/g}$ .

Palumbo *et al.* (1999) evaluated the efficiency of recovery of microorganisms from surface of swine carcasses by excision and swabbing techniques. The mean coliforms count of carcasses by one-site swab method was  $0.19 \pm 0.41 \log \text{ cfu}/\text{cm}^2$  whereas, the three site swab method yielded a mean coliforms count of  $0.28 \pm 0.87 \log \text{ cfu}/\text{cm}^2$ .

Yu *et al.* (1999) determined the microbiological quality of dehaired swine carcasses selected at random from different stages of operations. The mean number of coliforms on carcasses before first polishing was  $1.75 \log_{10} \text{ cfu}/\text{cm}^2$ . The count on carcasses after second singeing, before and after chilling was 0.66, 0.92 and  $0.54 \log_{10} \text{ cfu}/\text{cm}^2$ , respectively.

Gill and Jones (2000) compared the efficiency of recovery of bacteria from commercial pig carcasses by excision, and swabbing with cellulose acetate sponge, medical gauze and cotton wool on two groups of carcasses leaving spray cooling process. The total number of coliforms recovered from samples collected from one of the groups by excision, swabbing with sponge, gauze and cotton wool was  $1.78 \log_{10} \text{ cfu}/250 \text{ cm}^2$ ,  $4.01 \log_{10} \text{ cfu}/2500 \text{ cm}^2$ ,  $2.77 \log_{10} \text{ cfu}/2500 \text{ cm}^2$  and  $1.40 \log_{10} \text{ cfu}/125 \text{ cm}^2$ , respectively. The corresponding count of samples belonging to the

other group was  $1.89 \log_{10} \text{ cfu}/250 \text{ cm}^2$ ,  $2.73 \log_{10} \text{ cfu}/2500 \text{ cm}^2$ ,  $4.19 \log_{10} \text{ cfu}/2500 \text{ cm}^2$  and  $1.95 \log_{10} \text{ cfu}/125 \text{ cm}^2$ , respectively.

Gill *et al.* (2000a) assessed the microbiological conditions of pig carcasses each obtained from processing plants after polishing, dressing and cooling. Polished carcasses obtained from plants A, B, C, D, E and F had a mean coliforms count of 1.38, 1.06, 1.72, 2.77, 2.13 and  $1.32 \log_{10} \text{ cfu}/100 \text{ cm}^2$ , respectively. The count in the finally washed carcasses obtained from the plants A, B, C, D and E was 3.04, 2.08, 1.58, 2.35 and  $1.90 \log_{10} \text{ cfu}/100 \text{ cm}^2$ , respectively.

Gill *et al.* (2000b) evaluated the coliforms count of two sets of pig carcasses, each leaving the dressing process at a small abattoir. The mean coliforms count of carcasses belonging to one of the sets was  $4.27 \log_{10} \text{ cfu}/100 \text{ cm}^2$  and the count in the other set was  $3.51 \log_{10} \text{ cfu}/100 \text{ cm}^2$ .

Rivas *et al.* (2000) examined the level of microbial contamination on pig carcass samples collected after bleeding, scalding, dehairing, scraping, evisceration and at the end of line. The study revealed that the mean coliforms count on the carcasses after evisceration and at the end of line was  $1.18 \pm 0.84 \log \text{ cfu}/\text{cm}^2$  and  $1.39 \pm 0.98 \log \text{ cfu}/\text{cm}^2$ , respectively. The study also included the coliforms count of unwashed non-GMP carcasses, unwashed GMP carcasses and washed GMP carcasses. The mean coliforms count in unwashed non-GMP carcasses was  $1.21 \pm 0.74 \log \text{ cfu}/\text{cm}^2$ . The count on unwashed GMP carcasses and washed GMP carcasses was  $0.25 \pm 0.45$  and  $0.14 \pm 0.32 \log \text{ cfu}/\text{cm}^2$ , respectively.

Castelo *et al.* (2001a) evaluated the microbial decontaminating effect of various treatments on lean and fat trims of pork loins. The mean coliforms population on untreated lean pork trims was  $4.51 \pm 0.48 \log_{10} \text{ cfu}/\text{cm}^2$  and the count on untreated fat pork trims was  $4.37 \pm 0.18 \log_{10} \text{ cfu}/\text{cm}^2$ .

Castelo *et al.* (2001b) assessed the microbial attributes of ground pork prepared from pork trim subjected to different treatments. The total coliforms population in untreated ground pork samples was  $3.54 \log \text{ cfu}/\text{g}$ .



Gill and Badoni (2001) assessed the microbiological effects of pasteurisation treatment and subsequent trimming of sticking wound on microbiological condition of sticking wound area in pig carcasses. The mean coliforms count on carcasses before pasteurisation was  $0.92 \log \text{cfu/cm}^2$ . The total number of coliforms recovered after pasteurisation and trimming were 1.04 and  $1.99 \text{ cfu}/2500 \text{ cm}^2$ , respectively.

Gill *et al.* (2001) evaluated the recovery efficiency of bacteria by excision and swabbing with cotton wool, cellulose acetate sponge and gauze on fat and lean pork loins. The mean coliforms count on fat loin samples collected by excision, swabbing with cotton wool, sponge and gauze was 2.23, 2.22, 1.68 and  $1.14 \log_{10} \text{cfu}/100 \text{ cm}^2$ , respectively. The corresponding count of samples collected from lean loins was 2.30, 2.29, 1.90 and  $1.50 \log_{10} \text{cfu}/100 \text{ cm}^2$ , respectively.

Hansson (2001) evaluated the microbiological quality of pork carcasses in high and low capacity slaughter houses. The highest value of coliforms on pork carcasses from high capacity abattoirs was  $345 \text{ coliforms/cm}^2$  and that of the low capacity abattoirs was  $5500 \text{ coliforms/cm}^2$ .

Yu *et al.* (2001) evaluated the efficiency of recovery of bacteria from jowl area of prechill and post chill swine carcasses by excision and sponge swabbing methods. The pre chilled carcasses had a mean coliforms count of  $1.35 \log \text{cfu/cm}^2$  in the former method and  $0.70 \log \text{cfu/cm}^2$  by latter method. The samples taken from post chill carcasses by excision and swabbing method had count at the level of  $-0.05$  and  $-1.00 \log \text{cfu/cm}^2$ , respectively.

Erdmann *et al.* (2002) compared the efficiency of m-coli blue 24 (mCB) and violet red bile agar (VRBA) medium in recovering coliforms from pork samples collected by sponge swabbing technique. The coliforms count in the former medium was  $7.4 \text{ cfu}/15 \text{ cm}^2$  and that of latter medium was  $6.1 \text{ cfu}/15 \text{ cm}^2$ .

### 2.1.3 *Escherichia coli* count

Scriven and Singh (1986) evaluated the microbial quality of minced pork and retail pork rump samples. The MPN *Escherichia coli* in minced pork samples was 678 cells per g and on pork rump was 11 cells per gram.

Gupta *et al.* (1987) assessed the bacteriological quality of fresh pork samples collected from modern, semi-modern and rural abattoirs and also from two retail shops. The mean MPN *Escherichia coli* of samples from modern abattoir was  $6.49 \log_{10}/g$ , whereas the count of samples from semi-modern and rural abattoirs was 3.51 and  $3.87 \log_{10}/g$ , respectively. The count of the samples belonging to one of the shops was  $7.07 \log_{10}/g$  and that of samples from the other shop was  $6.07 \log_{10}/g$ .

Gill and Bryant (1993) estimated the number of *Escherichia coli* on pig carcasses each leaving dehairing and polishing machines on six days, at two plants A and B. The level of *Escherichia coli* on carcasses leaving the dehairing equipment at plant A ranged between  $9.0 \times 10^1$  and  $8.9 \times 10^2$  cfu/cm<sup>2</sup> and the count on the carcasses leaving the equipment at plant B varied from  $4.3 \times 10^2$  to  $4.3 \times 10^3$  cfu/cm<sup>2</sup>. The count on the carcasses leaving the polishing machines at plants A and B ranged between 1 and 6 cfu/cm<sup>2</sup> and 6 and  $2.5 \times 10^1$  cfu/cm<sup>2</sup>, respectively.

Vorster *et al.* (1994) reported the mean MPN of *Escherichia coli* on shoulderham samples as  $1.2 \log_{10}/g$ .

Gill *et al.* (1995) evaluated the microbial quality of polished, uneviscerated pig carcasses. The numbers of *Escherichia coli* recovered from unpasteurised carcasses varied upto the level of 6.2 /cm<sup>2</sup>.

Gill and Jones (1997a) assessed the hygienic performance of selected operations followed during the production of pasteurized pig carcasses. None of the carcasses entering the processes revealed the presence of *Escherichia coli* whereas, the carcasses leaving the process had a mean *Escherichia coli* count of  $0.99 \log_{10}/100\text{cm}^2$ .

Gill *et al.* (1997) analysed the microbial quality of pig carcasses selected at random from those entering or leaving pasteurisation treatment during commercial operations. The total number of *Escherichia coli* recovered from the samples grouped into two sets at sites other than anal area, before pasteurisation were 158 and 72. The samples collected from the anal area belonging to first set had a mean count of  $3.98 \log_{10}/100\text{cm}^2$  and the count on the second set of carcasses was  $3.53 \log_{10}/100 \text{ cm}^2$ .

Gill and Jones (1998a) compared the efficiency of three swab sampling methods in recovering *Escherichia coli* on pig carcasses leaving the cooling process. The mean *Escherichia coli* count recovered by the methods A and B was  $0.24$  and  $-0.17 \log_{10}/24 \text{ cm}^2$  whereas, method C had a recovery rate of  $-0.27 \log_{10}/100 \text{ cm}^2$ .

Gill and Jones (1998b) evaluated the bacterial quality of two sets of pig carcasses. The samples from each pig carcass was collected at the neck site after polishing, cutting open throat and floor of mouth and pasteurisation. The *Escherichia coli* count in one of the sets of carcasses after polishing and cutting open throat and floor of mouth were  $1.85$  and  $2.31 \log/2500 \text{ cm}^2$ , respectively. The corresponding count on the second set of carcasses was  $2.03$  and  $2.44 \log/2500 \text{ cm}^2$ , respectively. However, the pasteurized carcasses were found free of the organism in both the sets.

Gill *et al.* (1998) analysed the effect of hot water pasteurisation on bacterial load of pig carcasses. The total number of *Escherichia coli* recovered from unsplit and split carcasses prior to pasteurisation was  $2.28$  and  $3.33 \log \text{ cfu}/2500 \text{ cm}^2$ , respectively. The organism could not be detected in unsplit and split pasteurised carcasses.

Laubach *et al.* (1998) analysed the microbiological characteristics of swine head meat samples. Based on 144 samples, the level of *Escherichia coli* was  $2.25 \pm 0.42 \log_{10} \text{ cfu/g}$ .

Waskar *et al.* (1998) reported that the mean *Escherichia coli* count of deboned pork samples was 3.276 log cfu/g and the count of minced meat was 3.859 log cfu/g.

Palumbo *et al.* (1999) compared swabbing and excision techniques in the recovery of bacteria from swine carcass surfaces. The mean *Escherichia coli* count obtained from carcasses by one site swab method was  $-0.24 \pm 0.33$  log cfu/cm<sup>2</sup>. The corresponding count yielded from carcasses by three site swabbing method was  $0.37 \pm 1.03$  log cfu/cm<sup>2</sup>.

Gill and Jones (2000) compared the efficiency of bacteria recovery by excision and swabbing methods using cellulose acetate sponge, medical gauze and cotton wool on two groups of pig carcasses leaving a spray cooling process. The total number of *Escherichia coli* from carcasses belonging to group one by the four methods at a plant were 1.28 log cfu/250 cm<sup>2</sup>, 2.73 log cfu/2500 cm<sup>2</sup>, 2.41 log cfu/2500 cm<sup>2</sup> and 0.85 log cfu/125 cm<sup>2</sup>, respectively. The corresponding count on carcasses belonging to the second group were 1.72 log cfu/250 cm<sup>2</sup>, 2.45 log cfu/2500 cm<sup>2</sup>, 4.04 log cfu/2500 cm<sup>2</sup> and 1.81 log cfu/125 cm<sup>2</sup>.

Gill *et al.* (2000a) assessed the hygienic attributes of cleaning, dressing and cooling operations of pig carcasses at eight packing plants. The total number of *Escherichia coli* recovered from polished carcasses at plants A, B, C, D, E, F, G and H were 2.32, 2.19, 2.83, 2.45, 2.45, 1.28, 0.48 and 2.06 log cfu/2500 cm<sup>2</sup>, respectively. The corresponding count of carcasses after final wash was 3.75, 2.78, 2.70, 2.43, 2.01, 1.23, 1.79 and 1.76 log cfu/1500 cm<sup>2</sup>.

Gill *et al.* (2000b) assessed the hygienic condition of carcasses of cattle, pigs, deer, bison, ostriches and emus at the end of dressing process. During the study, two sets of pig carcasses were tested and the mean *Escherichia coli* count of one of the sets of carcasses was 2.64 log cfu/100 cm<sup>2</sup> and that of the other set was 2.86 log cfu/100 cm<sup>2</sup>.

Riyas *et al.* (2000) assessed the level of microbial contamination of pig carcasses at different stages of processing. The mean *Escherichia coli* count on

carcass surface after bleeding, scalding, dehairing, scraping, evisceration and at end of line was  $3.36 \pm 0.45$ ,  $0.10 \pm 0.16$ ,  $0.45 \pm 0.42$ ,  $0.05 \pm 0.12$ ,  $1.06 \pm 0.98$  and  $1.16 \pm 0.97$  log cfu/cm<sup>2</sup>, respectively. The mean *Escherichia coli* count on unwashed non GMP carcasses, unwashed GMP carcasses and washed GMP carcasses was  $1.20 \pm 0.72$ ,  $0.24 \pm 0.43$  and  $0.13 \pm 0.34$  log cfu/cm<sup>2</sup>, respectively.

Castelo *et al.* (2001a) examined the microbial decontamination efficiency of different sanitizing agents on lean and fat trims of pork loins. The *Escherichia coli* levels on fat trims before treatment was  $4.26 \pm 0.19$  log<sub>10</sub> cfu/cm<sup>2</sup>.

Gill and Badoni (2001) evaluated the microbiological effect of pasteurising treatment and subsequent trimming of the sticking wound on pig carcasses. The study revealed that before pasteurisation the carcasses yielded *Escherichia coli* at levels of  $2.09$  log cfu/2500 cm<sup>2</sup> and the number of organisms on the carcasses after pasteurisation and trimming were  $0.85$  and  $1.56$  log cfu/2500 cm<sup>2</sup>, respectively.

Gill *et al.* (2001) assessed the bacterial recovery by excision and swabbing methods on sets of fat and lean pork loins. The total number of *Escherichia coli* recovered by excision, cotton wool, sponge and gauge swabbing from fat pork loins were  $2.46$ ,  $2.89$ ,  $2.03$  and  $1.43$  logcfu/2500 cm<sup>2</sup>, respectively. The corresponding count on lean pork loins was  $3.03$ ,  $2.48$ ,  $2.25$  and  $1.60$  log cfu/2500 cm<sup>2</sup>.

Hansson (2001) analysed the microbiological quality of pork carcasses obtained from high and low capacity slaughter houses. The highest presumptive *Escherichia coli* count of carcasses from the high capacity slaughter houses was  $315$  cfu/cm<sup>2</sup> and the count on the carcasses from low capacity slaughter houses was  $107$  cfu/cm<sup>2</sup>.

Tamplin *et al.* (2001) evaluated the changes in the incidence and level of salmonella species and *Escherichia coli* biotype1 on pork carcasses before and after slaughter. The average daily concentration of *Escherichia coli* on chilled carcasses ranged from  $0.08$  to  $60$  cfu/cm<sup>2</sup>, with an overall average of  $1.1 \pm 7.1$  cfu/cm<sup>2</sup>.

Erdmann *et al.* (2002) compared the efficiency of recovery of *Escherichia coli* on pig carcasses by m-coli blue and EC petrifilm methods. The mean *Escherichia coli* count recovered by the former method was 4.0 log cfu/cm<sup>2</sup> and that of the latter method was 3.5 log cfu/cm<sup>2</sup>.

#### 2.1.4 Faecal streptococcal count

Borah *et al.* (1992) analysed the bacteriological quality of pork samples and reported the recovery of enterococci from the samples.

Knudtson and Hartman (1993) determined the number and species of enterococci present on pork carcasses during fabrication and subsequent processing. Samples were collected after singeing, polishing and final washing of the carcasses at the midpoint of loin and outside of ham. Of the 175 enterococci isolates recovered, 79 per cent were *Enterococcus faecalis*.

Wegener *et al.* (1996) examined the presence of vancomycin resistant *Enterococcus faecium* in retail pork samples and reported the isolation of the organism from 15 per cent of 26 pork samples.

Waskar *et al.* (1998) evaluated the microbiological profile of raw materials used in the manufacture of processed pork products. The mean faecal streptococcal count of deboned and minced pork samples was 4.386 log cfu/g and 5.313 log cfu/g, respectively.

Pavia *et al.* (2000) investigated the presence of vancomycin resistant enterococci in 100 meat samples. The study revealed that 33.3 per cent of the pork samples examined had *Enterococcus faecalis*.

Soriano *et al.* (2001) reported the incidence of various micro flora in lettuce, potato omelette and meat samples from restaurants. Enterococci were recovered from 12.5 per cent of the raw pork samples tested.

## 2.2 ISOLATION OF BACTERIA

### 2.2.1 Isolation of *Escherichia coli*

Nair *et al.* (1984) assessed the bacteriological quality of five native pig carcasses and reported the isolation of 60 *Escherichia coli*. Of these isolates, 56 belonged to *Escherichia coli* type I and four to type II. The serotypes isolated from the samples included O69, O146, O44, O129, O38, O1, O17, O13, O62, O20, O46, O55, O18, O6, O27, O11, O23, O41 and five isolates were identified as rough strains.

Scriven and Singh (1986) evaluated the microbial quality of retail pork rump and minced pork samples. The incidence of *Escherichia coli* in the former samples was 40 per cent and that in the latter samples was 90 per cent.

Doyle and Schoeni (1987) examined pork, beef, poultry and lamb samples to detect the presence of verocytotoxic *Escherichia coli* O157:H7. The organism was isolated from 1.5 per cent of pork samples.

Borah *et al.* (1988a) reported the isolation of 22 strains of *Escherichia coli* from pork which included the serotype O20, O2, O11, O52 and O61.

Borah *et al.* (1988b) studied the serotypes and drug sensitivity pattern of *Escherichia coli* isolated from pork samples. Of the 22 isolates, 12 belonged to serotypes O2, O3, O11, O20, O52, O61, O138 and O143, eight were untypable and two were rough strains.

Sulthienkul *et al.* (1990) examined pork, beef, chicken and vegetable samples collected from local markets and reported the isolation of *Escherichia coli*. Serotypes isolated from pork included O22, O101:H7, O76, O11:H2 and O146:H10 and one of the isolates produced Shiga like toxin.

Borah *et al.* (1992) evaluated the bacteriological quality of pork samples and reported the isolation of bacterial organisms. Of the isolates, *Escherichia coli* alone constituted 15.69 per cent.

Verotoxigenic *Escherichia coli* was isolated from two out of 13 pork samples examined (Condedara *et al.*, 1992).

Caprioli *et al.* (1993) assessed the role of farm animals as reservoir of verotoxin-producing *Escherichia coli* (VTEC). Of the five *Escherichia coli* isolated from pigs processed at different abattoirs, four isolates were verotoxin-producing *Escherichia coli* and belonged to serogroup O101.

Samadpour *et al.* (1994) examined ground beef, ground lamb, ground veal and pork samples and reported that four per cent of pork samples produced Shiga like toxin I (SLTI), six per cent produced Shiga like toxin II (SLTII) and one per cent produced both the toxins.

Sharma *et al.* (1995) tested 350 samples of raw milk, meat and meat products collected from local markets and retail shops and reported the isolation of *Escherichia coli* from 7.69 per cent of pig muscle samples. The serotypes isolated from the samples included O9, O91 and O164.

Heuvelink *et al.* (1996) assessed the performance of 3M Petrifilm Test Kit-HEC to detect the occurrence of *Escherichia coli* in retail raw meat samples. The organism was isolated from only one of the minced pork samples.

Gill and Jones (1997b) evaluated the hygienic performance of a spray cooling process on pig carcasses entering and leaving the process and reported that none of the samples had *Escherichia coli*.

Gill and Jones (1998a) assessed the efficiency of sampling pig carcasses with sponge, single gauze swab and three swab methods in the recovery of *Escherichia coli*. Out of the 252 samples swabbed by sponge, 61 had *Escherichia coli*. However, out of 275 carcasses, the organism was isolated from 66 samples by single swab and from 82 samples by three swab methods.

Gill and Jones (1998b) examined the mouth and surfaces of 40 pig carcasses to detect the presence of *Escherichia coli*. The organism was isolated from the



mouth of all carcasses and 10 per cent of these isolates were verotoxigenic *Escherichia coli* O157:H7 strains.

Korsak *et al.* (1998) evaluated the efficiency of a gauze swabbing technique as a reliable and easy method for monitoring the microbial quality of pig carcass surfaces. Of the half carcasses examined, *Escherichia coli* O157:H7 was isolated from 14 per cent of the samples.

Waskar *et al.* (1998) reported that *Escherichia coli* was present in 75 per cent of deboned pork samples.

Heuvelink *et al.* (1999a) reported the isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from slaughter pigs and poultry. *Escherichia coli* O157 strains were isolated from 1.4 per cent of pigs.

Heuvelink *et al.* (1999b) evaluated 262 raw pork samples in 1996 and 207 samples in 1997, to determine the presence of verocytotoxin-producing *Escherichia coli* O157. The samples examined in 1996 had the organism in 0.8 per cent and none of the samples tested in 1997 yielded the organism.

Palumbo *et al.* (1999) compared the efficiency of excision and swabbing techniques in determining the microbiological quality of swine carcass surfaces. A total of 30 carcasses were swabbed at ham, belly and jowl areas and *Escherichia coli* was detected in 15 ham, four belly and three jowl samples.

Rheault and Quessey (1999) evaluated the microbial contamination of stick wounds in swine carcasses. It was found that 27.7 per cent of the stick wounds had *Escherichia coli* and after trimming, 26.2 per cent of the samples showed the presence of the organism.

Gill *et al.* (2000a) evaluated the hygienic performance of cleaning, dressing and cooling process' of pig carcasses at eight packing plants. The fraction of *Escherichia coli* in the coliforms recovered from polished pig carcasses at the eight plants viz., A, B, C, D, E, F, G and H were 64, 40, 56, 12, 18, 5, 18 and 86 per cent, respectively.

ZhiJiang *et al.* (2000) reported the presence of *Escherichia coli* O157 in one of the pork samples examined.

Banerjee *et al.* (2001) examined several foods of animal origin including five pork samples for the presence of verotoxin-producing *Escherichia coli* (VTEC). The organism was isolated from 40 per cent of the samples. None of the isolates were verotoxic.

Bouvet *et al.* (2001) reported the occurrence of verotoxin-producing *Escherichia coli* and *Escherichia coli* O157 in pig carcasses from three slaughterhouses. Study revealed that 50 per cent of the carcass samples were VTEC positive and none of the samples yielded *Escherichia coli* O157:H7. Skin samples of belly, leg and shoulder allowed the detection of more than 80 per cent of VTEC positive strains.

Shiga toxin producing *Escherichia coli* was isolated from four per cent of the retail pork samples tested (Brooks *et al.*, 2001).

Duffy *et al.* (2001) determined the extent of microbiological contamination in retail pork samples. They reported that *Escherichia coli* was found in 1.3 per cent of the samples examined.

Hansson (2001) assessed the microbiological quality of pork carcasses at high and low capacity slaughterhouses. Presumptive *Escherichia coli* was observed in 74 per cent of the pork samples taken from high capacity abattoirs whereas, 58 per cent of the samples from low capacity abattoirs had the organism.

Leung *et al.* (2001) determined the prevalence and characterisation of verotoxin-producing *Escherichia coli* isolated from cattle and pigs from an abattoir. VTEC was isolated from 0.2 per cent of porcine faecal and carcass samples.

Rho *et al.* (2001) investigated the microbial hazards at swine farms, slaughterhouses and processing lines and reported that none of the pork samples had *Escherichia coli* O157.

Soriano *et al.* (2001) assessed the incidence of *Escherichia coli* in 40 raw pork samples and the organism was isolated from four samples. None of the samples revealed the presence of *Escherichia coli* O157:H7.

Tamplin *et al.* (2001) examined the presence of *Escherichia coli* biotype I on swine carcasses processed under HACCP- based inspection model project. *Escherichia coli* was found in all the 100 pre-eviscerated carcasses and an average of 30.1 per cent of chilled carcasses.

Bouvet *et al.* (2002a) evaluated the effect of cutting process on pork meat contamination by verotoxin-producing *Escherichia coli* (VTEC) and of *Escherichia coli* O157:H7. VTEC was isolated from 12 per cent of carcasses, 19 per cent of primary cuts and five per cent of secondary cuts. However none of the samples yielded *Escherichia coli* O157.

Bouvet *et al.* (2002b) assessed the effect of slaughtering process on pig carcass contamination by *Escherichia coli* O157.VTEC faecal carriage was observed in 31 per cent.

Bouvet *et al.* (2002c) detected the presence of verotoxin-producing *Escherichia coli* (VTEC) and *Escherichia coli* O157:H7 among pig carcass and pork samples. Verotoxin-producing *Escherichia coli* (VTEC) was recovered from 26 per cent of carcass samples and 12 per cent of pork samples from cutting plants.

### 2.2.2 Isolation of *Staphylococcus aureus*

Vanderzant and Nickelson (1969) examined the microbiological quality of beef, pork and lamb carcasses and reported that coagulase positive staphylococci were isolated from 59 per cent of the ham samples.

Dasgupta and Chandrachoudhury (1975) reported the isolation of coagulase positive staphylococci from 30 per cent of pig meat samples.

Pandurangarao (1977) reported the isolation of coagulase positive staphylococci from 14 out of 55 pork samples.

Prasad *et al.* (1979) evaluated pork and pork products collected from bacon factory and retail shops and reported the isolation of pathogenic staphylococci from 10 out of 25 pork samples.

Nkanga and Uraih (1981) reported that 17.5 per cent of raw pork samples had *Staphylococcus aureus*.

Nair *et al.* (1984) evaluated the bacteriological quality of pig carcasses and reported the presence of *Staphylococcus aureus* as about 240 to 800 organisms per gram of pork.

Bachhil (1985) evaluated the presence of certain bacterial pathogens in fresh, processed and frozen meat and reported that 11.1 per cent of the pork samples had *Staphylococcus aureus*.

Scriven and Singh (1986) evaluated the incidence of *Staphylococcus aureus* in minced pork and beef and also pork and beef rump samples and reported that the incidence of coagulase positive staphylococci in minced pork and pork rump samples was 50 per cent and 30 per cent, respectively.

Schraft *et al.* (1991) analysed swab specimens collected from pig hindquarters used for cured raw ham products and reported that 22.7 per cent of the samples was contaminated with *Staphylococcus aureus*.

Shimizu *et al.* (1991) reported the isolation of *Staphylococcus aureus* from meat and raw fish samples. The organism was recovered from 26 per cent of pork samples.

*Staphylococcus aureus* was isolated from 24.18 per cent of market pork samples (Borah *et al.*, 1992).

Isigidi *et al.* (1992) studied the enterotoxin production in different *Staphylococcus aureus* biotypes isolated from various food sources including minced pork samples. Thirty of the 185 strains examined, revealed the presence of one or more enterotoxins.

Hua and Ling (1994) reported the presence of *Staphylococcus aureus* in 105 meat samples including 30 pork samples.

Vorster *et al.* (1994) estimated the bacteriological quality of shoulderham samples and reported the frequency of occurrence of *Staphylococcus aureus* in 6.7 per cent of the samples.

Gill *et al.* (1995) determined the bacteriological quality of polished uneviscerated carcasses subjected to hot water treatment (85°C for 2s). The study revealed that staphylococci constituted two percent of the microflora isolated from pig carcasses.

Saide-Albornoz *et al.* (1995) evaluated the level of pork carcass contamination during slaughter, fabrication and subsequent storage. They reported the isolation of *Staphylococcus aureus* as 4.4, 7.4 and 12.6 percent from ham and loin surface samples after singeing and polishing, final rinse and 24h chill, respectively.

Desai and Kamat (1998) reported the isolation of three coagulase positive staphylococci in meat based foods, including pork.

Waskar *et al.* (1998) reported the isolation of *Staphylococcus aureus* from 50 per cent of deboned pork and 62.5 per cent of minced pork samples.

Coagulase positive staphylococci were detected in 49 and 16 per cent of pork carcasses from high and low capacity abattoirs respectively (Hansson, 2001).

Rho *et al.* (2001) studied the microbiological hazards at farms, slaughterhouses and processing lines of swine for the application of HACCP system in Korea. They reported that *Staphylococcus aureus* was the most frequently detected pathogen in slaughterhouses and processing rooms.

Soriano *et al.* (2001) evaluated the microflora on a total of 370 samples including lettuce, beef, pork, chicken and Spanish potato omelette from restaurants and reported that 17.5 per cent raw pork samples had *Staphylococcus aureus*.

Son (2002) reported the isolation, toxin production and virulence of *Staphylococcus aureus* in pork samples collected from abattoirs and butcher shops. Out of the isolates, 72 per cent were ST toxinogenic, 27.27 per cent were LT toxinogenic and 38 per cent were both ST and LT toxinogenic.

### 2.2.3 Isolation of *Salmonella*

Bachhil (1985) evaluated the sanitary indices and quantified certain pathogenic organisms in pork, mutton, chevon, fresh and frozen sausages. They reported that none of the pork samples yielded salmonellae.

Morgan *et al.* (1985) reported the isolation of *Salmonella* species from 35 out of 80 jowl samples examined and the isolates belonged to the serotypes, *Salmonella anatum*, and *Salmonella give*.

Gupta and Chauhan (1986) evaluated the bacteriological quality of fresh pork and pork products and reported the isolation of *Salmonella* from fresh pork samples.

*Salmonella* was not isolated from any of retail pork rump and minced pork samples (Scriven and Singh, 1986).

Gupta *et al.* (1987) analysed the bacteriological quality of pork samples collected from three types of abattoirs and two retail shops. The study revealed that 12 per cent of fresh pork samples had *Salmonella* and the serotypes of the isolates were *Salmonella bareilly* and *Salmonella stanley*.

Yadava *et al.* (1988) assessed the dynamics of contamination of pork with *Salmonella* in a processing plant and reported that 2.43 per cent of pork samples was contaminated with *Salmonella london*.

Mafu *et al.* (1989) reported the incidence of *Salmonella* in swine carcasses and slaughterhouse environment. They reported the isolation of *Salmonella derby* from 1.5 per cent of diaphragm samples.

Sinell *et al.* (1990) examined minced pork samples which had salmonellae and stored the samples at  $-18^{\circ}\text{C}$  for one to 14 weeks. Only 131 samples revealed the presence of the organisms on retesting, after the storage period.

Bhattacharya *et al.* (1991) tested pigs slaughtered for human consumption and reported the isolation of *Salmonella paratyphi A* from one of the samples.

Borah *et al.* (1992) evaluated the bacteriological quality of pork samples and reported that *Salmonella* was not isolated from any of the samples.

Paturkar *et al.* (1992) tested meat samples that consisted of beef, mutton, pork and chicken samples and reported the isolation of *Salmonella anatum* and *Salmonella derby* from pork samples.

Gill and Bryant (1993) examined the presence of *Escherichia coli*, *Salmonella* and *Campylobacter* species on pig carcasses passing through dehairing equipment and reported that *Salmonella* was recovered from none of the samples.

Escartin *et al.* (1995) reported the isolation of 34 *Salmonella* serovars from raw pork samples and the most frequent serovars were *Salmonella agona*, *Salmonella derby*, *Salmonella anatum*, *Salmonella meleagridis*, *Salmonella enteritidis*, *Salmonella worthing*, *Salmonella give*, *Salmonella manhattan*, *Salmonella typhimurium* and *Salmonella brandenburg*.

Sáide-Albornoz *et al.* (1995) evaluated the prevalence of five types of pathogenic bacteria on pork carcasses during slaughter, fabrication and refrigerated storage. *Salmonella* was isolated from 4.4 per cent of carcasses after singeing and polishing, 1.1 per cent of the samples after final rinse, 0.4 per cent of carcasses after 24h chill and 0.7 per cent of boneless loins after packaging.

Fehlhaber *et al.* (1996) reported the mean incidence of *Salmonella* as 1.7 per cent on swine carcasses.

Kuri *et al.* (1996) examined the hygienic quality of pork samples purchased from randomly selected sites. A total of 51 salmonellae were isolated from the

samples which belonged to the serotypes *Salmonella derby*, *Salmonella anatum*, *Salmonella bredeney*, *Salmonella agona*, *Salmonella heidelberg*, *Salmonella muenster*, *Salmonella worthington*, *Salmonella saint-paul*, *Salmonella muenchen*, *Salmonella typhimurium*, *Salmonella brandenburg*, *Salmonella give*, *Salmonella infantis*, *Salmonella senftenburg*, *Salmonella eko*, *Salmonella havana*, *Salmonella lockleaze*, *Salmonella new-brunswick*, *Salmonella orion* and *Salmonella roterberg*.

Berends *et al.* (1997) quantified the risk factors associated with *Salmonella* contamination on pork carcasses and reported that five to 15 per cent of carcasses revealed the presence of the organism during polishing after singeing.

Lazaro *et al.* (1997) examined tonsils and lymph node samples collected from swine carcasses. Of the 31 tonsil samples, 39 isolates of salmonellae were recovered and the predominant serotype (93.5%) was *Salmonella muenster*. The other serotypes included *Salmonella infantis*, *Salmonella fyris* and *Salmonella derby*. The organism was also isolated from 32 lymph node samples. Of the 34 isolates, 31 (96.9%) were *Salmonella muenster*.

Miller *et al.* (1997) assessed the effect of buying source, feed withdrawal before slaughter and the incidence of punctured GI tracts during evisceration on the number of pathogenic and spoilage bacteria on pork carcasses. The study revealed that none of the samples yielded salmonellae.

Berends *et al.* (1998) reported the incidence of *Salmonella* in contaminated primal cuts and retail pork samples as 25-30 per cent whereas, in minced pork and sausages the organism was present in about 50-55 per cent samples.

Carr *et al.* (1998) analysed the effect of chilling and trimming on the microbial populations of pork carcasses and reported that one of the samples had *Salmonella*.

Korsak *et al.* (1998) evaluated the efficiency of a swabbing technique in the recovery of foodborne pathogens from pork carcasses at four slaughterhouses. Of the half carcasses assayed, 27 per cent yielded *Salmonella*.



Laubach *et al.* (1998) evaluated the level of microflora on swine headmeat samples. The incidence of salmonellae isolated from meat samples of cheek, back of head and tongue was 3.8, 4.3 and 5.8 per cent, respectively.

Waskar *et al.* (1998) conducted a study to evaluate the microbial profile of various raw, semiprocessed products and ingredients used in pork processing. They reported that 12.5 per cent of raw minced pork samples had salmonellae.

Duffy *et al.* (1999) examined pork samples and reported the isolation of *Salmonella* from two samples which belonged to the serotypes, *Salmonella bredeney* and *Salmonella typhimurium*.

Kalimuddin and Choudhary (1999) tested the presence of *Salmonella* in raw pork samples and reported the isolation of the organism from 12 per cent samples and the isolates were *Salmonella agona*, *Salmonella weltevreden*, *Salmonella berta* and *Salmonella emek*.

Szadados and Szadados (1999) conducted a study on the incidence of *Salmonella* in pig carcasses during bacteriological meat inspection. They reported that the organism was isolated from carcasses and the most commonly occurred pig adapted serovar was *Salmonella choleraesuis* (22%) and *Salmonella typhisuis* (20.3%).

Giovannacci *et al.* (2001) investigated the sources of contamination of *Salmonella* in pig carcasses and cuts in two different plants. They reported the isolation of eight serotypes of *Salmonella* after killing, dehairing, polishing and splitting which included *Salmonella goldcoast*, *Salmonella concord*, *Salmonella bredeney*, *Salmonella typhimurium*, *Salmonella derby*, *Salmonella brandenburg*, *Salmonella infantis* and *Salmonella london*.

Ludenig *et al.* (2001) examined the swine carcasses from four finishing pig producers. They reported that *Salmonella* incidence ranged from two to eight per cent and *Salmonella typhimurium* was the predominant serotype (58.8%).

Rho *et al.* (2001) studied the microbial hazards at swine farms, slaughterhouses and processing lines and reported that salmonellae were not detected in any of the samples collected at different occasions.

Soriano *et al.* (2001) conducted a study to determine the various types of microorganisms in different foods and reported that the raw pork samples examined were free from salmonellae.

Swanenburg *et al.* (2001a) reported that the prevalence of *Salmonella* in pork samples of seronegative herds was lower than in samples of seropositive herds.

Swanenburg *et al.* (2001b) reported the prevalence of *Salmonella* on swine carcasses as 1.4 per cent and the predominant serotype isolated was *Salmonella typhimurium*.

Pamplin *et al.* (2001) examined the prevalence of various bacterial species on slaughtered pigs. *Salmonella* was detected on 73 per cent of post exsanguinated pigs and on 0.7 per cent chilled carcasses.

Thorberg and Engvall (2001) conducted a study on the incidence of *Salmonella* on pork carcasses collected from five Swedish slaughterhouses and reported that no *Salmonella* was detected from any of the samples.

Bolton *et al.* (2002) examined the impact of washing and chilling pork carcasses in enhancing foodsafety control under HACCP system. The study revealed that after bleeding 50 per cent of samples had *Salmonella* and after powerhosing the organism was detected from seven per cent of the samples. The isolates belonged to the serotypes *Salmonella typhimurium* and *Salmonella agona*.

Korsak *et al.* (2003) monitored the contamination points in a closed pig production chain, to develop a *Salmonella* free production system. *Salmonella* was detected in 11.2 per cent of 52 carcass swab samples and the predominant serotype was *Salmonella typhimurium*.

#### 2.2.4 Isolation of *Listeria monocytogenes*

Wong *et al.* (1990) examined a variety of foods for the incidence of *Listeria monocytogenes* and reported the isolation of the organism from 58.8 per cent of pork samples.

Buncic (1991) reported that 45 per cent of all slaughtered pigs examined had *Listeria monocytogenes* in their tonsils and three percent were faecal excretors. The organism was also found in 69 per cent of minced pork and beef samples.

Simon *et al.* (1992) investigated the incidence of *Listeria monocytogenes* in minced pork, beef and poultry samples and reported that 17.3 per cent of the minced meat had the organism.

Wang *et al.* (1992) examined meat samples that consisted of pork, beef, lamb and chicken, to determine the contamination of retail meat with *Listeria monocytogenes*. The organism was isolated from seven of 22 pork samples.

Loncarevic *et al.* (1994) evaluated 496 samples that consisted of meat, lymph nodes, process water and swabs from different parts of an abattoir and reported that four pig meat and one lymph node of pigs had *Listeria monocytogenes*

Nesbakken *et al.* (1994) evaluated the effect of sealing rectum on the reduction of spread of *Yersinia* and *Listeria* species on pig carcasses and reported that *Listeria monocytogenes* was not detected in any of the samples taken from pig carcasses, each from Norway or Sweden. However the organism was recovered from 33 per cent of carcasses eviscerated without rectal sealing

Adesiyun and Krishnan (1995) sampled pigs slaughtered at an abattoir in Trinidad for the occurrence of *Yersinia*, *Listeria* and *Campylobacter* species at different sites. The study revealed that only 1.9 per cent of carcass samples yielded *Listeria monocytogenes* and the organism was also isolated from five per cent of rectal samples.

Gill and Jones (1995) examined the presence of pathogens on skinned pork loins and processing equipment at two pig slaughtering plants. *Listeria monocytogenes* was isolated from 23 of 24 loin samples at Plant A and from only one of the loin samples at Plant B.

Saïde-Albornoz *et al.* (1995) evaluated the bacterial contamination of pork carcasses during slaughter, fabrication and chilled storage. *Listeria monocytogenes* was detected in 1.5 per cent of the samples after singeing and polishing and in 1.9 per cent of the samples after final wash and 24h chilled storage.

Takeshige *et al.* (1995) studied the epidemiological pattern of *Listeria monocytogenes* occurrence on dressed pig carcasses and reported the isolation of the organism from the intestinal contents (0.4%) and body surfaces (0.3%)

Yu-*et al.* (1995) estimated the presence of *Listeria* species in raw meats. Out of the 20 ground pork samples, *Listeria* species was isolated from seven samples, with *Listeria monocytogenes* occurring in five samples.

Loncarevic *et al.* (1997) reported a case of human foodborne listeriosis and tested 23 food items including pork samples, collected from the patient's refrigerator, for the presence of *Listeria monocytogenes*. Of the food items, opened packages of sliced pork brawn and sliced cooked medwurst had the organism.

Miller *et al.* (1997) assessed samples including pork carcasses to determine the incidence of various pathogens and reported that *Listeria monocytogenes* was not detected from the samples tested.

Korsak *et al.* (1998) evaluated the efficiency of swabbing beef and pork carcasses with sterile gauze, to detect *Salmonella*, *Campylobacter*, *Listeria monocytogenes* and verocytotoxin producing *Escherichia coli*. The study revealed that five of the 49 pork carcasses examined had *Listeria* species, one of which was *Listeria monocytogenes*.

*Listeria monocytogenes* was isolated from 20.5 per cent of mechanically deboned pork, 16.4 per cent of pork trimmings and 6.9 per cent of pork loins and hindleg samples (Samelis and Metaxopoulos, 1999).

Autio *et al.* (2000) evaluated the *Listeria monocytogenes* contamination pattern in low capacity slaughterhouses. The organism was recovered from six of 50 carcass samples.

Bæk *et al.* (2000) reported the incidence and characterisation of *Listeria monocytogenes* from different domestic and imported foods. The organism was detected from 19.1 per cent of pork samples.

Chasseignaux *et al.* (2001) monitored the origin of *Listeria monocytogenes* in pork and poultry meat collected from two processing plants and reported that eight out of 24 pork samples revealed the presence of the organism.

Rho *et al.* (2001) investigated on the various microbial hazards at swine farms, slaughterhouses and processing lines and reported that *Listeria monocytogenes* was not detected in any of the eviscerated pork carcasses.

Kanauganti *et al.* (2002) surveyed 300 hogs and pork products like ground pork and raw chitterlings for *Listeria monocytogenes*. The study revealed that 45 per cent of 340 ground pork samples had the organism and 1.5 per cent had other *Listeria* species.

Peccio *et al.* (2003) reported the occurrence and characterisation of seven strains of *Listeria monocytogenes* on pork samples obtained from a swine meat processing plant.

#### 2.4 BACTERIAL COUNT ON ENVIRONMENT AND PROCESSING EQUIPMENTS

Mafu *et al.* (1989) made an investigation to determine the incidence of contamination of cooler-ready hog carcasses by *Salmonella* spp. *Campylobacter* spp. and *Yersinia enterocolitica*. They reported that *Salmonella* species were

isolated from 10 per cent of the samples. The distribution of salmonellae isolated from slaughter floor was 8.9 per cent and cold room floor, 4.4 per cent. *Campylobacter* species was isolated from 61.7 per cent of 400 specimens. *Yersinia enterocolitica* was isolated from 9.3 per cent of 448 specimens.

Rao and Ramesh (1992) analysed water, knives, floor, wall, processing equipments and hands of workers before and after slaughtering operations to identify the critical points of microbial contamination in the slaughter line. The study indicated that the total viable count of samples before slaughtering operation was at the level of  $10^2$  cfu/cm<sup>2</sup> or per ml in the floor washings, wall and equipments. The mean count in water and knives samples was at the level  $10^1$  cfu/cm<sup>2</sup> or per ml. They observed a four fold increase of the count in the samples collected after slaughter.

Gill and Bryant (1993) tested the presence of certain microorganisms, in pig carcass dehairing equipment at two plants, A and B. The detritus from the machines at both plants yielded bacteria at total numbers between  $8.5 \times 10^7$  and  $9.3 \times 10^8$  cfu/g, *Escherichia coli* at numbers between  $1.9 \times 10^4$  and  $1.1 \times 10^5$  cfu/g and *Campylobacter* at numbers between  $5.2 \times 10^3$  and  $1.1 \times 10^6$  cfu/g. *Salmonella* was recovered from about 50 per cent of the samples at numbers between  $3 \times 10^3$  and  $3.7 \times 10^5$  cfu/g at plant A and  $1 \times 10^2$  cfu/g at plant B. The total numbers and numbers of *Escherichia coli* and *Campylobacter* in the waters in the two machines that operated in series at plant A at temperature less than 50°C, were respectively, between  $1.7 \times 10^5$  and  $1.3 \times 10^6$ ,  $5.5 \times 10^2$  and  $1.1 \times 10^3$  and  $4.5 \times 10$  and  $8 \times 10^2$  cfu/ml. The corresponding count in the water from plant B were respectively, between  $3.5 \times 10^4$  and  $1.3 \times 10^5$ ,  $1.4 \times 10^3$  and  $5.3 \times 10^3$  and  $2 \times 10$  and  $5 \times 10^2$  cfu/ml. *Salmonella* was recovered from about 50 per cent of the water samples at both plants at numbers between  $7 \times 10$  and  $5.5 \times 10^2$  cfu/ml at plant A and  $1 \times 10$  cfu/ml at plant B.

Tarwate *et al.* (1993) evaluated the potential source of microbiological hazards associated with the slaughter line operations and the surrounding circumstances. They examined knife, axe, saw-blade, hooks, floor, wall platform,

hand swab and water and reported that the total viable count in the samples varied from  $2.9 \log_{10} \text{ cfu/cm}^2$  to  $6.7 \log_{10} \text{ cfu/cm}^2$ . The coliforms count ranged between  $4.2 \log_{10} \text{ cfu/cm}^2$  and  $6.9 \log_{10} \text{ cfu/cm}^2$ . Water samples revealed the lowest total viable count and the mean count of the samples was  $2.07 \log_{10} \text{ cfu/ml}$ . 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.

Karr *et al.* (1996) estimated the bacterial count of twenty cutting table and conveyor belt surface swab samples and reported that APCs on the conveyor surfaces were lower with all samples under  $10^3 \text{ cfu/cm}^2$  and 53.7 per cent under  $50 \text{ cfu/cm}^2$ . The total coliforms count of cutting tables and conveyor belts was  $\leq 10^2 \text{ cfu/cm}$  or lower and 44 per cent had count of less than one  $\text{cfu/cm}^2$ . For count of *Escherichia coli* biotype I, 51 per cent of conveyor surface samples had count of less than  $10 \text{ cfu/cm}^2$  and 46 per cent had count of less than one  $\text{cfu/cm}^2$ .

Eisel *et al.* (1997) evaluated the microbial load of air, floor and processing equipments in a red meat processing plant. Sanitised processing equipments had a mean total viable count of  $1 \text{ cfu/cm}^2$  and the count on processing floors was  $5 \text{ cfu/cm}^2$ . Coliforms and *Escherichia coli* were rarely recovered from the source. The total viable count of the air samples was generally low ( $0.6 \text{ cfu/m}^3$ ) and the count in the carcass receiving area was  $2.4 \text{ cfu/m}^3$ .

Lazaro *et al.* (1997) analysed scalding tank water and environmental swab samples of two abattoirs for the presence of *Salmonella* species. The organism was recovered from one out of ten (10 per cent) scalding tank water samples, 45.5 per cent of evisceration table swabs, 20 per cent of butchering saw swab samples, 30 per cent of samples from killing room and 20 per cent of samples from holding pen, at abattoir B. None of the environmental samples from abattoir A was positive for *Salmonella*.

Rahkio and Korkeala (1997) studied the microbiological contamination of air in four slaughter houses by using impactor samples taken from back splitting

and weighing area. The mean aerobic count of the air in the former area was 2.25 log<sub>10</sub> cfu/100 litres of air and the count in the latter area was 2.03 log<sub>10</sub> 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 conveyer belt support bars had a mean total aerobic count of two to five log<sub>10</sub> cfu/cm<sup>2</sup> and the count on steel mesh gloves was seven to nine log<sub>10</sub> cfu/cm<sup>2</sup>. No bacteria was recovered from samples of counter weighed saw and cutting boards. Coliforms and *Escherichia coli* count on steel mesh gloves was greater than 3.0 log<sub>10</sub> cfu/cm<sup>2</sup>.

Autio *et al.* (2000) assessed the *Listeria monocytogenes* contamination pattern in 10 pig slaughter houses. The organism was recovered from 10 per cent of swab samples on tables and 20 per cent of saw swab samples. None of knife swab samples yielded the organism.

Rivas *et al.* (2000) studied the microbial contamination of equipments in an industrial slaughterhouse. The mean APC and Enterobacteriaceae count of knife and knife sharpener was 3.22 ± 1.22, 0.88 ± 1.12 and 3.81 ± 0.41 and 2.15 ± 0.85 log cfu/cm<sup>2</sup> respectively. The mean APC and Enterobacteriaceae count for dirty equipment (after 3h work) namely dehairing table, dehairing machine blades and scraping machine whips was 5.80 ± 0.87, 4.39 ± 0.87 and 6.16 ± 0.99 log cfu/cm<sup>2</sup>, respectively. The corresponding count on the equipments after cleaning was 3.77 ± 1.30, 4.10 ± 0.97 and 6.51 ± 0.78 log cfu/cm<sup>2</sup>.

Thorberg and Engvall (2001) evaluated the incidence of *Salmonella* in five Swedish slaughter houses and reported that none of environmental samples examined which consisted of water from scalding tank, polisher machine and carcass splitter swabs had the organism.



## 2.5 STANDARDS FOR BACTERIAL COUNT

The level and type of bacterial contamination on the carcass 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 meat products.

### 2.5.1 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) ICMSF (1986) recommended that the total viable count for fresh meats at  $35^{\circ}\text{C}$  should be less than  $10^7$  per gram (Gracey *et al.*, 1999).

### 2.5.2 *Escherichia coli* count

Carl (1975) suggested the *Escherichia coli* limit for freshmeat 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) USDA (1996) stipulates the microbiological criteria for *Escherichia coli* on pig carcasses as that a process fails to meet the criterion if in the 13 samples most recently collected, four or more samples yield *Escherichia coli* at numbers greater than  $10\text{ cfu/cm}^2$  but less than or equal to  $10,000\text{ cfu/cm}^2$  or one or more samples yield *Escherichia coli* at numbers greater than  $10,000\text{ cfu/cm}^2$  (Gill, 2000).

### 2.5.3 *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) ICMSF (1986) suggested that *Salmonella* should not be detected in more than one of five 25 g of meat samples (Gracey, 1999). USDA

(1996) suggested the microbiological criteria for salmonella on pig carcasses which indicated that a process fails to meet the criterion, if more than six out of 55 pig carcasses are positive for the organism (Gill, 2000). According to the Food act, Government of Mauritius (1998) *Salmonella* should be absent in 25 g of raw meat.

#### **2.5.4 *Listeria monocytogenes***

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

# *Material and Methods*

### 3. MATERIALS AND METHODS

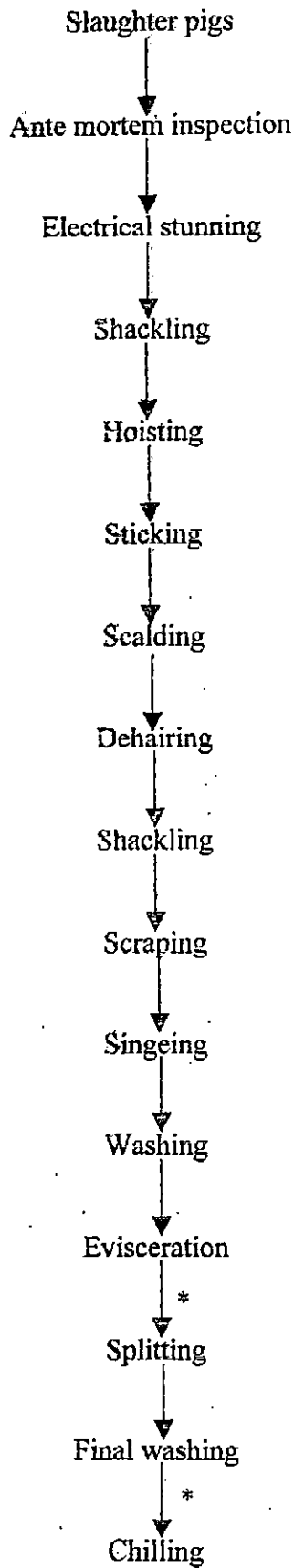
The present study was undertaken to evaluate the bacterial quality of pork carcasses after evisceration and final washing in the slaughterline of a meat processing plant located at Kochi in Kerala. The slaughter and dressing of carcasses in the factory consist of antemortem inspection, stunning, shackling, sticking, hoisting, scalding, dehairing, washing, singeing, washing, evisceration, splitting, final washing and chilling (Flow chart 1). In the present investigation, 25 pork carcasses were randomly selected. In order to obtain a representative sample of each carcass, a total of 500cm<sup>2</sup> area was sampled from one of the sides of the carcass, immediately after evisceration, and an equivalent area from the other side after final washing.

#### 3.1 COLLECTION OF SAMPLES

On each visit, two carcasses were selected and sampled. The samples were collected from the eviscerated carcasses by swabbing an area of 100 cm<sup>2</sup> each from jowl, shoulder, bacon, loin and ham (Plate. 1). Each area was marked with a sterile aluminium template of 10cmx10cm (inside area) and swabbed with a sterile cotton swab (Hansson, 2001) of about 2.5 cm breadth, hydrated with 0.1% peptone water (diluent) and the excess diluent in the swab was removed by gentle pressing of swab against the sides of sterile diluent containing conical flask. The marked areas were swabbed lengthwise with one side of the swab, breadth wise with the other side and corner to corner by the tip of the swab. After swabbing, an area, the swab was transferred into a flask containing 100ml sterile diluent and brought to the laboratory in a thermocool container. Samples from other half of the carcasses were collected after final washing and transported to the laboratory as described above (Flow chart 2).

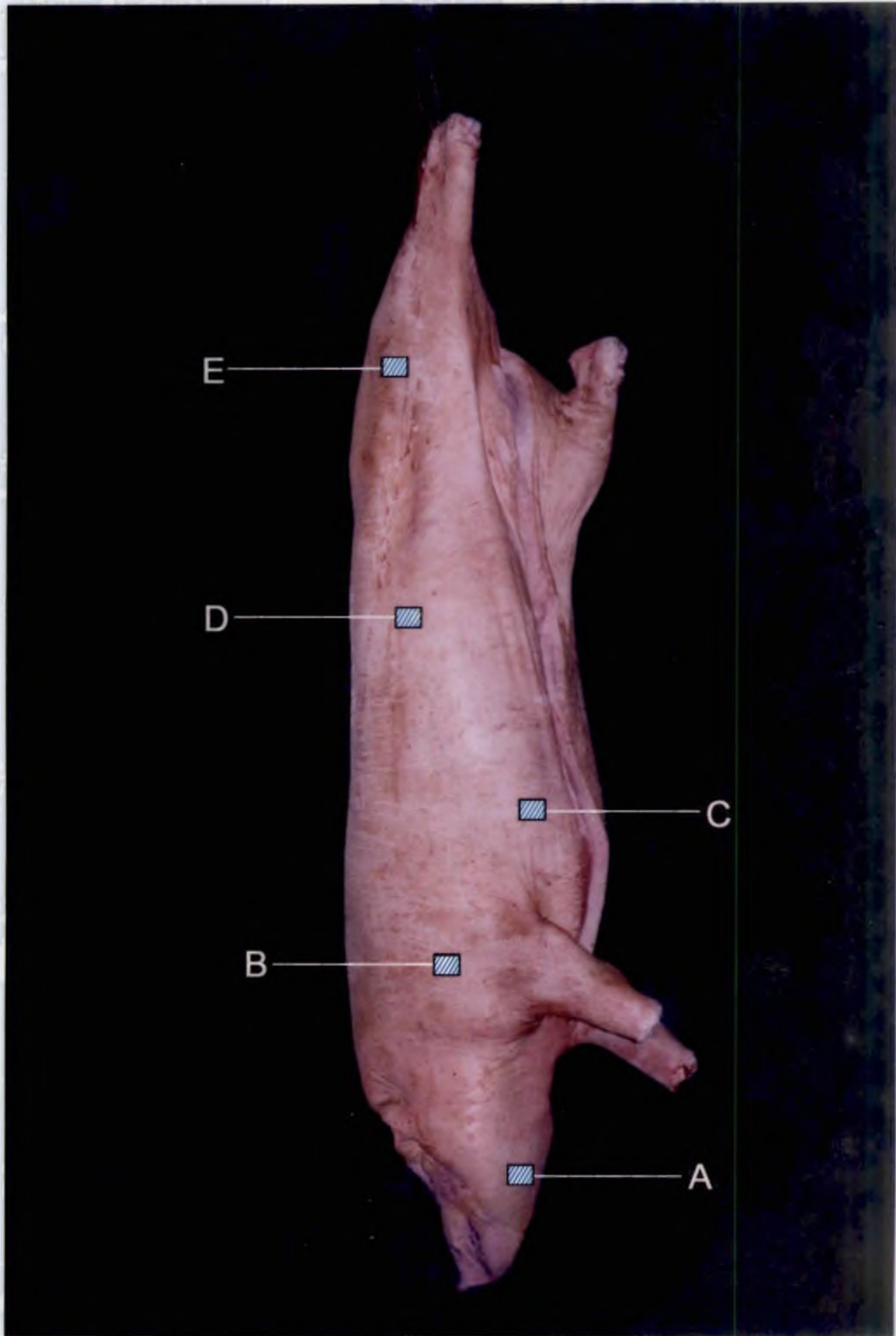
#### 3.2 PROCESSING OF SAMPLES

Each swab was cut at length with a pair of sterile scissors and dropped into the diluent. The swab with the diluent was thoroughly agitated using a cyclomixer. The agitated five swab samples collected from each carcass after evisceration and

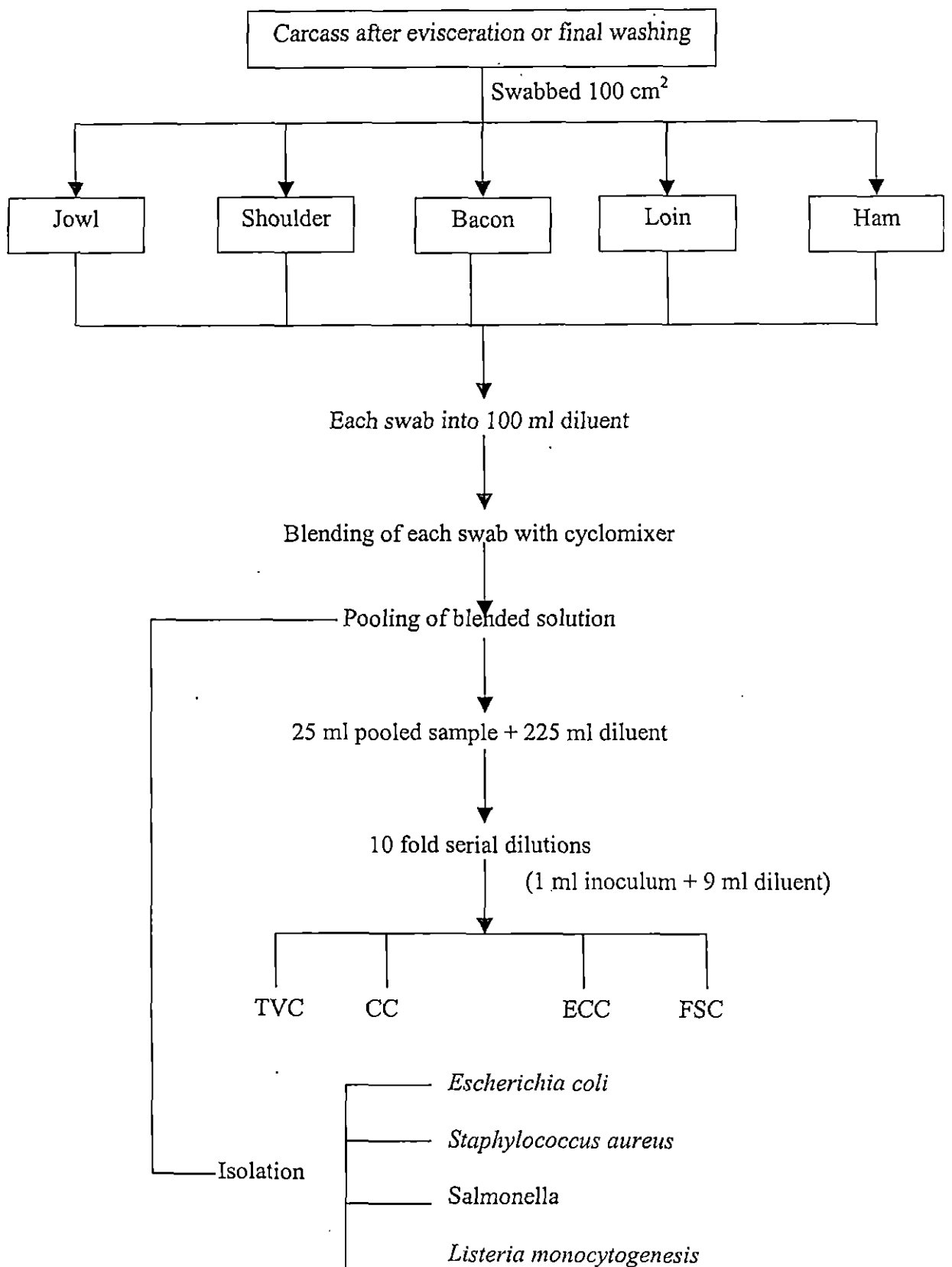


\* Sampling points  
*After evisceration*  
*After final washing*

**Flow chat 1. Slaughter and dressing of pork carcass**



**Plate 1: Sampling sites on pork carcass:  
(A) Jowl (B) Shoulder (C) Bacon (D) Loin (E) Ham**



**Flow chart 2. Collection and processing of samples**

after final washing were transferred to another conical flask so as to form the stock sample.

### 3.3 PREPARATION OF SAMPLES

In order to estimate the bacterial load of the samples collected from each carcass, either after evisceration or after final washing, 25 ml of the stock sample was transferred to 225 ml of diluent so as to form 1 in 10 dilution. From this further ten fold serial dilutions were made by transferring one millilitre of inoculum to nine millilitre of diluent. From each sample dilutions were made upto  $10^{-7}$ . Selected dilutions of each sample were used for the enumeration of total viable count (TVC), coliforms count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC) as shown in table 1. The stock samples prepared from each carcass, after evisceration and final washing were used for the isolation of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*

### 3.4 BACTERIAL COUNT

#### 3.4.1 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 on to 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 solidify at room temperature and were then incubated at 37°C for 24 h. At the end of incubation period, petri-dishes with a bacterial count between 30 and 300 colonies were selected and count of each petri-dish was taken with the help of a colony counter. The number of colony forming units (cfu) per  $\text{cm}^2$  of the carcass was calculated by multiplying the mean colony count of duplicate plates with dilution factor and the count was expressed as  $\log_{10}\text{cfu}/\text{cm}^2$ .



**Table 1. The techniques employed for the enumeration of various bacteria**

Type of Bacterial counts	Media	Quantity of inoculum (ml)	Incubation		Colony characteristics
			Temperature (°C)	Period (h)	
Total viable count	Standard Plate Count Agar	1	37	24	All colonies
Coliforms count	Violent Red Bile Agar	0.1	37	24	Purplish red colonies with diameter at least 0.5 mm, surrounded by a reddish zone of precipitate
<i>Escherichia coli</i> count	Eosine Methylene Blue Agar	0.1	37	24	Colonies with a dark centre giving a distinct indelible ink, greenish black metallic sheen on deflected light
Faecal streptococcal count	KF Streptococcal Agar	0.1	37	48	Pink to dark red colonies with a diameter varying between 0.5 to 3 mm and surrounded with a narrow whitish zone

### 3.4.2 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 on to 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 cm<sup>2</sup> of the carcass surface was estimated by applying the dilution factor on the mean count of duplicate plates and the count was expressed as log<sub>10</sub>cfu/cm<sup>2</sup>.

### 3.4.3 *Escherichia coli* count

The *Escherichia coli* count (ECC) per cm<sup>2</sup> of the carcass surface was estimated as prescribed by Indian Standards (1980). The count was estimated by inoculating 0.1 ml of the inoculum from selected dilution on to 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 center and a distinct indelible ink, greenish black metallic sheen on deflected light was counted as *Escherichia coli*. The number of organisms per cm<sup>2</sup> of carcass was estimated as described for CC and the count was expressed on log<sub>10</sub>cfu/cm<sup>2</sup>.

### 3.4.4 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 per cm<sup>2</sup> on carcass surface, 0.1 ml of the inoculum from the selected dilution was transferred on to 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 3 mm and surrounded by a narrow whitish zone were counted as faecal streptococci. The number of organisms per cm<sup>2</sup> of the carcass surface was estimated and expressed as described in CC.

### 3.5 ISOLATION AND IDENTIFICATION OF BACTERIA

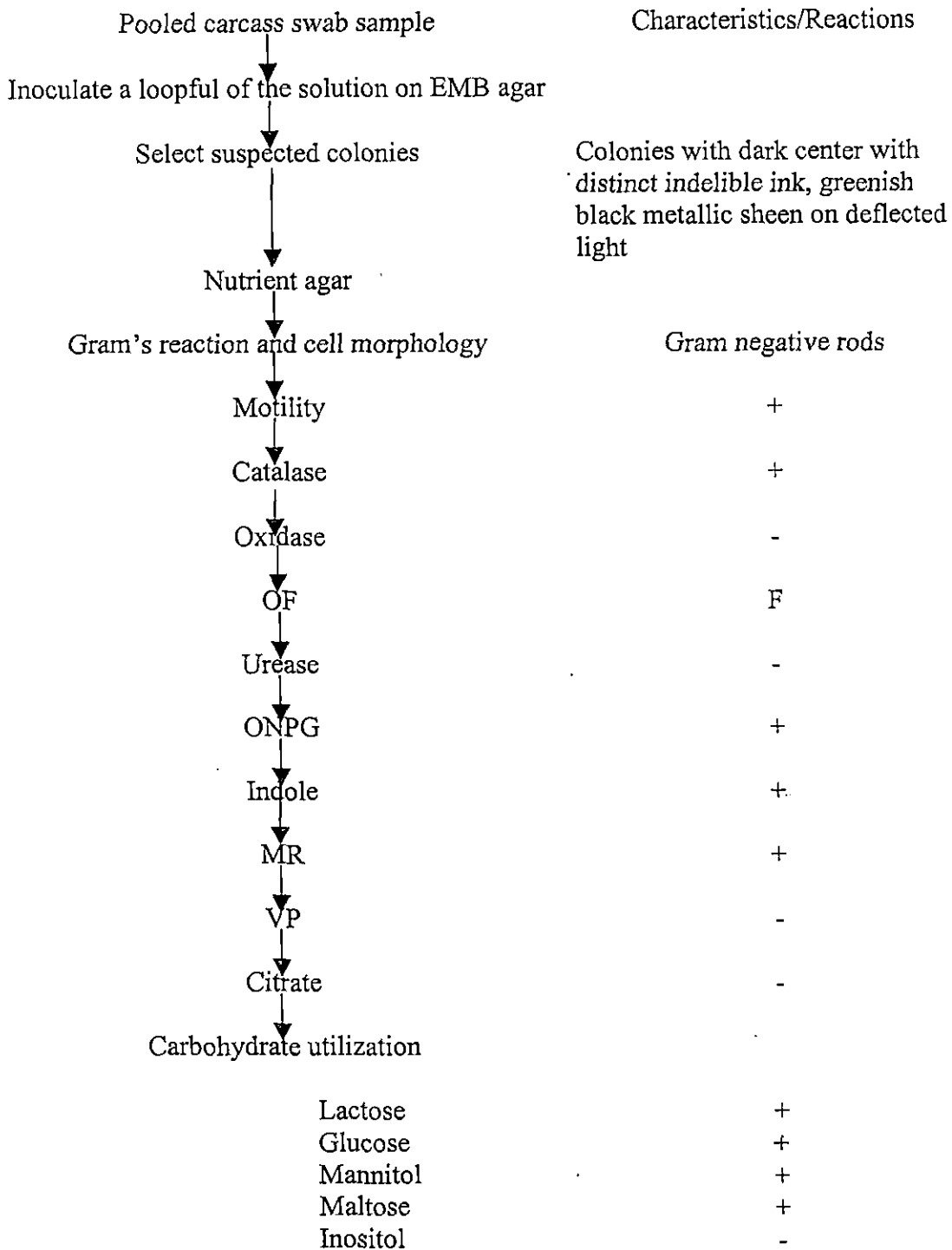
The stock solution prepared from each carcass, after evisceration and after final washing, was subjected to the isolation and identification of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*.

#### 3.5.1 *Escherichia coli*

To isolate *Escherichia coli*, a loopful of the stock solution was inoculated on to duplicate plates of Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. After the incubation period, three or four colonies with the characteristic of *Escherichia coli* were transferred on to nutrient agar slants and incubated at 37°C for 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 3. The isolates identified as *Escherichia coli* were serotyped at National Salmonella and Escherichia Center, Central Research Institute, Kasauli.

#### 3.5.2 *Staphylococcus aureus*

For the isolation of *Staphylococcus aureus*, a loopful of the stock solution was inoculated on to Baird-Parker (BP) agar medium and were incubated at 37°C for 48 h (AOAC, 1990). At the end of incubation, colonies showing characteristic appearance (circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, grey-black to jet-black, frequently with light-coloured (off-white) margin, surrounded by opaque zone (precipitate) and frequently with outer clear zone and with buttery to gummy consistency on BP agar medium were selected and transferred to nutrient agar slants and incubated at 37°C for overnight. The isolates were stored at refrigeration temperature for further characterization and



Nitrate  
test -ve

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

**Flow chart 3. Isolation and identification of *Escherichia coli* from pork carcass**

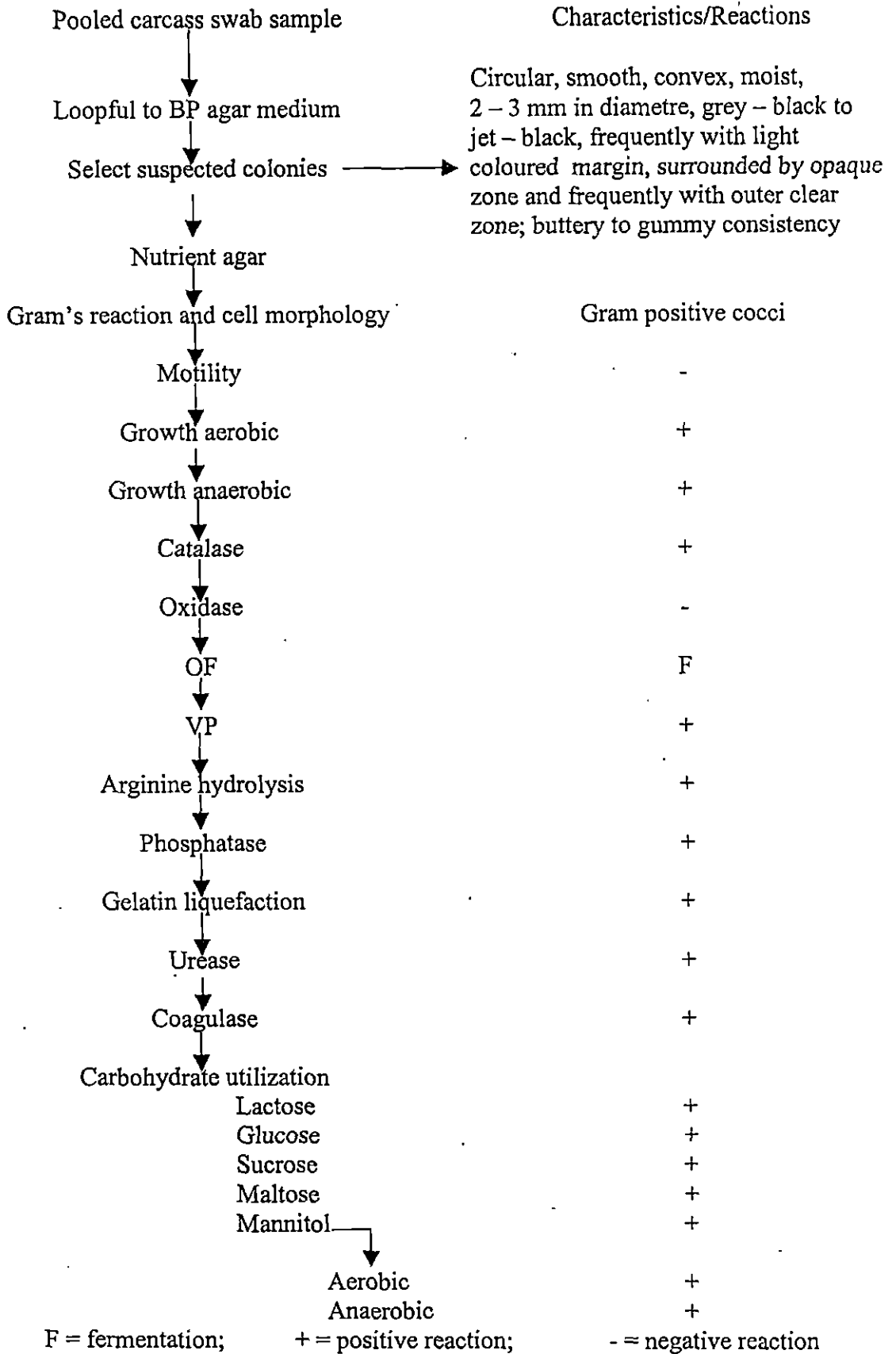
identification of the isolates following the procedure described by Barrow and Feltham (1993) and are shown in the flow chart 4. The isolates were identified based on the cultural, morphological and biochemical characteristics.

### 3.5.3 *Salmonella*

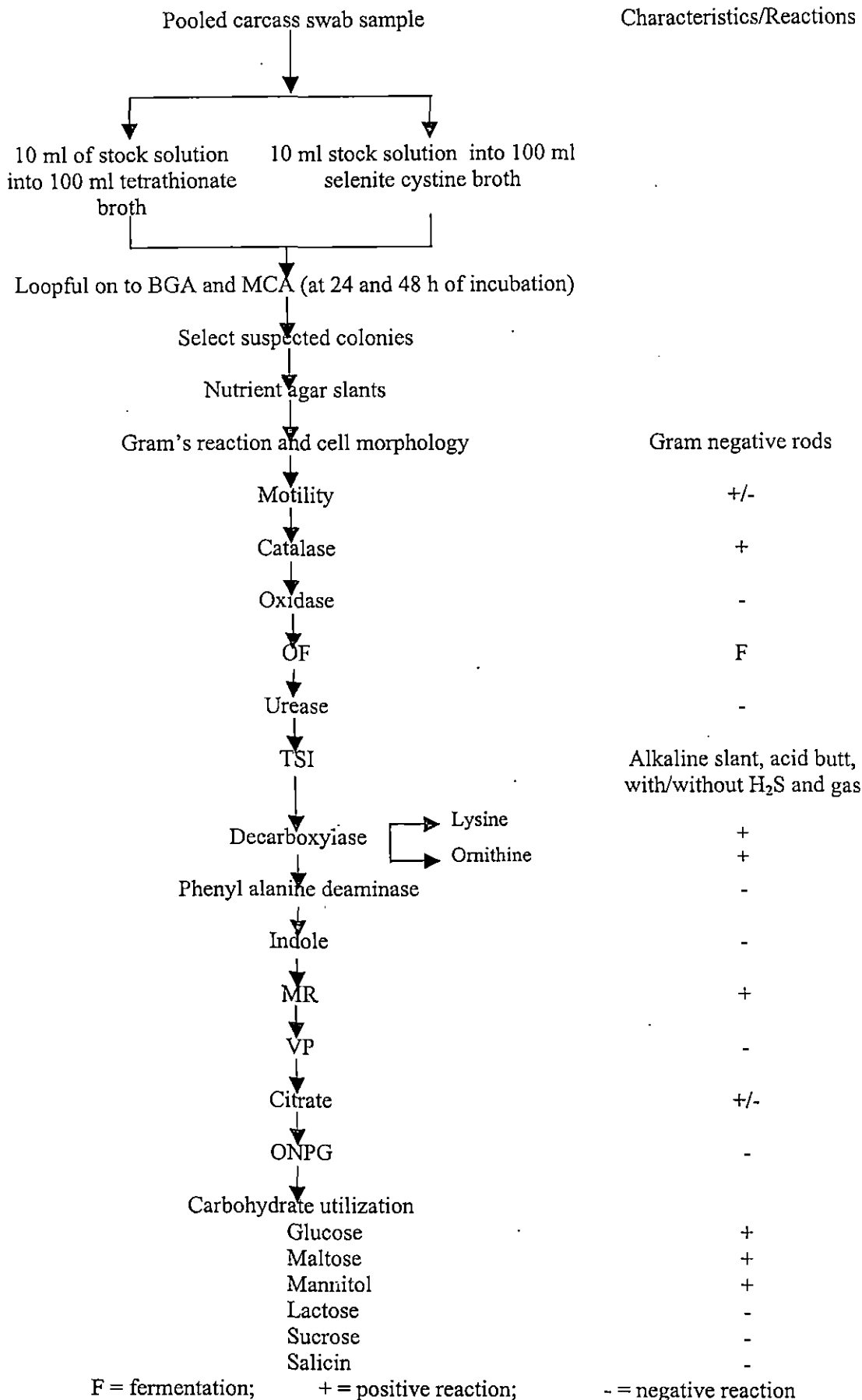
In order to isolate salmonellae from each carcass, 10 ml each of the stock solution was transferred into sterile conical flask containing 100 ml of tetrathionate broth (Hi-media) and an equal quantity of inoculum into 100 ml selenite cystine broth (Hi-media) (Konwar and Joshi, 2000). The contents of the flasks were mixed thoroughly and the former flask was incubated at 37°C for 48 h and the latter 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 on to duplicate plates of Brilliant Green Agar (BGA) (Hi-media) and MacConkey 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 1-2 mm, surrounded by a pink or red hue on BGA and transparent colourless colonies with opaque center on MCA were selected. The selected colonies were transferred to nutrient agar slants and incubated at 37°C for overnight and stored at refrigeration temperature for further characterization of the isolates. The cultural, morphological and biochemical characteristics of the isolates were identified according to the procedure described by Edwards and Ewing (1972) and Barrow and Feltham (1993) and are shown in flow chart 5.

### 3.5.4 *Listeria monocytogenes*

From the stock solution, 10 ml was transferred into a sterile conical flask containing 90 ml of *Listeria* enrichment broth (Autio *et al.*, 2000), mixed thoroughly and incubated at 30°C for 7 days. A loopful of the inoculum was streaked on to duplicate plates of Oxford agar and Polymyxin B Acriflavin Lithium Chloride Ceftazidime Aeseulin Mannitol (PALCAM) agar on the second and seventh day of incubation and incubated at 30°C for 24-48 h. At the end of incubation, the colonies with a black zones on Oxford agar and those with cherry



**Flow chart 4. Isolation and identification of *Staphylococcus aureus* from pork carcass**



**Flow chart 5. Isolation and identification of *Salmonella* from pork carcass**

red background on PALCAM agar were inoculated onto Lithium Chloride Phenylethanol Moxalactam (LPM) agar. The plates were incubated at 30°C for 24 h and examined under Henry's oblique lighting technique. Typical grayish blue colonies were picked and inoculated onto Trypticase Soy Agar (TSA) slants and incubated at 30°C for 24 h and stored at 4°C for further characterization of the isolates. The isolates were subjected to various tests prescribed by Wang et al. (1992) as shown in the flow chart 6. The isolates showing characteristics similar to that in flow chart were considered as *Listeria monocytogenes*.

### 3.6 CHARACTERISATION AND IDENTIFICATION OF ISOLATES

The isolates were identified by the following tests.

#### 3.6.1 Primary identification tests

##### 1. Catalase test

###### *Slide test*

A small quantity of the colony was transferred on to a clean, grease free, glass slide and mixed well with a drop of three per cent hydrogen peroxide. Evolution of effervescence within a few seconds indicates a positive reaction (Barrow and Feltham, 1993).

###### *Tube test*

One ml of three per cent hydrogen peroxide solution was poured over the slope of a nutrient agar slant on which the isolate was grown. A positive reaction is indicated by the development of effervescence immediately (Barrow and Feltham, 1993).

##### 2. Gram staining

The procedure for gram staining is as follows:





- a. A thin smear of each isolate was made on a clean, grease free glass slide. Air dried the smear and then heat fixed by passing over a flame.
- b. The smear was then flooded with 0.5 per cent crystal violet in water and allowed to act for 30 seconds.
- c. Poured off the stain and washed with water.
- d. Flooded the smear with Gram's iodine solution (one per cent iodine and two per cent potassium iodide in water) for 30 seconds.
- e. Poured off the solution and the smear was decolourised with a few drops of acetone and allowed to act for two to three seconds.
- f. Washed the smear and counterstained 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 (Barrow and Feltham, 1993)

### **3. Motility test**

*Motility of the organism was assessed by stabbing the isolate into the Hugh and Leifson's medium with a straight wire upto a depth of 5 mm. Motility was indicated by a spreading growth into the medium from the line of inoculation and growth of non motile organisms is confined to the stab (Barrow and Feltham, 1993).*

### **4. Oxidase test**

A filter paper strip is moistened with a few drops of an aqueous solution of one per cent tetramethyl-para-phenylenediamine dihydrochloride. Each isolate was then smeared across the paper strip with a platinum loop. The appearance of a dark purple colour on the paper strip within 30 seconds indicates a positive reaction (Barrow and Feltham, 1993).

## **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 centimetres above the medium. The tubes were incubated at 37°C for upto 14 days. A change in colour of the medium from green to yellow in the open tube alone is taken as oxidation whereas a change in colour from green to yellow in both the tubes is regarded as fermentation. Absence of colour change in both tubes indicate no action on carbohydrates (Barrow and Feltham, 1993).

### **3.6.2 Secondary tests**

#### **1. Aesculin hydrolysis**

The organism was inoculated into aesculin broth and was incubated at 37°C and examined daily for five days. Blackening of the broth due to hydrolysis of aesculin indicates a positive reaction (Barrow and Feltham, 1993).

#### **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 indicated by the development of a brown colour. (Barrow and Feltham, 1993).

#### **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 indicates acid production and the production of gas is indicated by the appearance of air bubbles in the inverted Durham's tube. Anaerobic condition of the medium was provided by adding a

layer of sterile molten soft paraffin to a depth of about one centimeter above the media (Barrow and Feltham, 1993).

#### **4. Citrate utilization test**

A light suspension of the organism was made in normal saline and was inoculated with a straight wire on to the slope of Simmon's citrate agar. The inoculated medium was incubated at 37°C and examined daily up to seven days. The ability of the organism to utilize citrate as the sole source of carbon is indicated by a change in colour of the medium from green to blue and growth of the organism along the streak line (Barrow and Feltham, 1993).

#### **5. Coagulase test**

##### *Slide test*

A small quantity of the culture was emulsified in a drop of saline on a microscope slide to produce a thick suspension. The suspension was stirred with a straight wire dipped in rabbit plasma. A positive result is indicated by macroscopic clumping within five seconds and delayed clumping is considered as negative reaction (Barrow and Feltham, 1993).

##### *Tube test*

Mixed 0.5 ml undiluted rabbit plasma with an equal volume of an 18 to 24 h broth culture of the test organism and incubated at 37°C and examined after one and four hours for coagulation. Negative tubes were left at room temperature overnight and re-examined (Barrow and Feltham, 1993).

#### **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 is taken as the control. The organism was inoculated through the paraffin layer and incubated at 37°C for five days. In a

positive reaction, the medium first turns yellow and then becomes purple and the control tubes remain yellow (Barrow and Feltham, 1993).

### **7. Eijkman test**

Each test organism was inoculated into tubes containing MacConkey broth with inverted Durham's tube, warmed to 37°C and incubated at  $44 \pm 0.1^\circ\text{C}$  in a water bath for 48 h. Production of both acid and gas indicates a positive reaction (Barrow and Feltham, 1993).

### **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 two hours and then examined for liquefaction. A positive result is indicated by liquefaction of gelatin (Barrow and Feltham, 1993).

### **9. Hippurate hydrolysis**

The slope of hippurate agar was lightly inoculated with the test organism and examined daily for seven days. Hydrolysis of hippurate is indicated by growth and the development of a pink colour due to alkali production (Barrow and Feltham, 1993).

### **10. Indole production**

The isolate was inoculated into peptone water and incubated at 37°C for 48 h. At the end of incubation added 0.5 ml of Kovac's reagent, mixed well and examined. A red colour in the reagent layer indicates a positive reaction (Barrow and Feltham, 1993).

### **11. Methyl red (MR) reaction**

The MR-VP medium was inoculated with the isolate and incubated at 37°C for two days. Added two drops of methyl red solution at the end of incubation

period and examined. Development of a red colour indicates positive reaction (Barrow and Feltham, 1993).

## **12. ONPG (O-nitrophenyl- $\beta$ -D-galactopyranoside) test**

Each isolate was inoculated into ONPG broth and incubated at 37°C for 48 h. The  $\beta$  galactosidase activity of the organism is indicated by the development of an yellow colour due to the production of O-nitrophenol (Barrow and Feltham, 1993).

## **13. Phenyl alanine deamination**

The phenyl alanine agar slope was heavily inoculated with the test organism and incubated at 37°C for overnight. At the end of incubation, 0.2 ml of 10 per cent aqueous solution of ferric chloride was poured over the slope. A positive result is indicated by the development of a green colour on the slope and in the free liquid at the base (Barrow and Feltham, 1993).

## **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 over it. Free phenolphthalein liberated by phosphatase reacts with the ammonia and phosphatase positive colonies become bright pink (Barrow and Feltham, 1993).

## **15. Triple sugar iron test**

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

## 16. Urease activity

Slopes of Christensen's urea agar was heavily inoculated with the test organism and incubated at 37°C. The tubes were examined after four hours of incubation and daily for five days. Development of a red colour in the medium indicates a positive reaction. (Barrow and Feltham, 1993).

## 17. Voges-Proskauer reaction

The MR-VP medium inoculated with the isolate was subjected to methyl red test. After completion of the test, added 0.6 ml of 5%  $\alpha$  naphthol solution and 0.2 ml of 40% aqueous potassium hydroxide into the tube. After thorough mixing of the contents, the tube was kept in a slanting position and examined after 15 minutes and one hour. A positive reaction is indicated by the development of a strong red colour (Barrow and Feltham, 1993).

## 3.7 ENUMERATION OF BACTERIA FROM SAMPLES OF ENVIRONMENT, PROCESSING EQUIPMENTS AND HAND WASHINGS OF PERSONNEL

### 3.7.1 Air

Direct exposure method described by Evancho et al. (2001) was employed for the enumeration of air borne microorganisms in different areas of pig slaughter line. To estimate the bacterial counts in the air, duplicate petri-dishes containing sterile nutrient agar medium was exposed for 15 minutes. The plates were brought to the laboratory in thermocool container and incubated at 37°C for 48 hours. The number of colonies developed in the duplicate plates was counted and the mean count was expressed as colony forming units (cfu) / min.

### 3.7.2 Water

#### 3.7.2.1 *Collection of samples*

##### a. Pond water

A pond, about 500 m away from the abattoir formed the source of water supply for the various operations in the abattoir. A clean sterile bottle of 250 ml

capacity was held by its bottom and plunged its neck downwards below the surface of water. The bottle was then turned until the neck pointed slightly upwards. When filled with water, the bottle was raised rapidly above the surface of water and then stoppered. The samples were brought to the laboratory in a thermocool container.

#### **b. Tap water**

Samples were collected from a tap in regular use at the slaughter hall of the abattoir. Allowed the water from tap run to waste for about two minutes to flush the interior of the nozzle and discharge the stagnant water. In order to, collect the sample a sterile bottle of 250 ml capacity, was held near the base with one hand and filled from a gentle stream of water from the tap, avoiding splashing.

#### **c. Scalding water**

The temperature of the scalding tank water was measured during the processing of pig 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 thermocool container.

#### **d. Hand wash of personnel**

On each visit, the hands of an individual were washed in 100 ml of 0.1 per cent sterile peptone water and the washing was collected into a sterile conical flask and stoppered. The samples were brought to the laboratory in a thermocool container.

#### **3.7.2.2 Processing of samples**

Prepared 1 in 10 dilution of the sample by adding 10 ml of sample to 90 ml sterile quarter strength Ringer's solution and mixed vigorously. One milliliter of the inoculum was transferred to 9 ml diluent so as to form serial 10 fold dilutions upto  $10^{-9}$ . Selected serial dilution of each water sample was used to estimate TVC, CC and ECC.



All the water samples were subjected to bacteriological examination by the method described by ISI (1978).

### **3.7.3 Processing equipments**

#### **a. Cutting table and cutting board**

For sampling meat cutting table and meat cutting board, a sterile cotton swab was moistened in 0.1 per cent sterile peptone water and the excess solution was pressed out against the interior wall of the vial with a rotating motion. The swab head was rubbed slowly and thoroughly over 100 cm<sup>2</sup> surface, which was marked with a sterile template (10 x 10 cm<sup>2</sup> internal area). The swab was rubbed three times, reversing direction between strokes. The swab head was cut with sterile scissors, transferred into a sterile vial and brought to the laboratory in a thermocool container.

#### **b. Knife and steel**

In order to collect samples of knife and steel the swab was moistened with 0.1 per cent sterile peptone water and it was slowly moved firmly three times over the surface, reversing the direction at each time. After swabbing, the processing and transportation of the swab was carried out as described in sampling of cutting table and board.

#### **c. Bell scraper and carcass splitter**

To collect samples from the bell scraper and carcass splitter, the moistened swab was moved over the surface of the equipments several times by reversing the direction of the swab. After swabbing, the processing and transportation of the swab was carried out as described in sampling of cutting table and board.

#### **d. HDPE pouches**

Twenty millilitre of 0.1 per cent sterile peptone water was poured into each of 500g capacity pouches and agitated ten times by holding vertically and

horizontally. The samples were then collected into a sterile conical flask by keeping the pouches upright.

All samples of environment, processing equipments and hand washings of personnel were collected six times.

#### **3.7.4 Evaluation of bacterial load**

The preparation, processing and estimation of bacterial load on the swab samples collected from the processing equipments and HDPE pouches was carried out as in the case of carcass swab stock solution.

#### **3.8 STATISTICAL ANALYSIS**

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

# Results

## 4. RESULTS

During the investigation, samples collected from carcasses after evisceration and after final washing. Samples from these carcasses were subjected to estimate the level of various bacteria per  $\text{cm}^2$  of the carcass surface and also for the isolation and identification of certain bacteria of public health significance. Processing equipments, environmental samples and hand washing of personnel engaged in the dressing line of the plant were also tested to detect the level of different bacterial population.

### 4.1 BACTERIAL COUNT

All samples collected from both eviscerated and finally washed carcasses were tested to determine the bacterial quality of the carcasses by estimating the total viable count, coliforms count, *Escherichia coli* and faecal streptococcal count.

#### 4.1.1 Total viable count

The mean total viable count (TVC) of the samples collected from the carcasses after evisceration and after final washing is given in table 2. The data of

**Table 2. Mean total viable count of the eviscerated and finally washed pork carcasses**

Samples		Mean $\pm$ SE ( $\log_{10}$ cfu/ $\text{cm}^2$ )
No.	Stage of dressing	
25	AE	5.14 <sup>a</sup> $\pm$ 0.16
25	AFW	5.31 <sup>a</sup> $\pm$ 0.16

P<0.05, AE - After evisceration, AFW - After final washing

Figures bearing the same superscript in the same column differ significantly

the samples were subjected to analysis of variance test, which showed a significant (P<0.05) difference between the mean count of the samples from eviscerated and

finally washed carcasses. The higher mean count was found in the finally washed carcasses.

#### 4.1.1.1. Distribution of carcasses based on the level of total viable count

The distribution of eviscerated and finally washed carcasses based on the level of total viable count per  $\text{cm}^2$  is shown in table 3 & fig. 1. Samples collected from carcasses after final washing had count at the level of  $10^3$  to  $10^6$  cfu/ $\text{cm}^2$  while the samples collected from the carcasses after evisceration had count only upto  $10^5$  cfu/ $\text{cm}^2$ . In 64 per cent of eviscerated carcasses, the count was at the level of  $10^5$  cfu/ $\text{cm}^2$  whereas, only 56 per cent of finally washed carcasses showed the count at the above level. Only four per cent of the samples of the latter group had count at the level of  $10^3$  cfu/ $\text{cm}^2$  while the count at the level was seen in 16 per cent of the carcasses after evisceration.

**Table 3. Distribution of carcasses based on the level of total viable count**

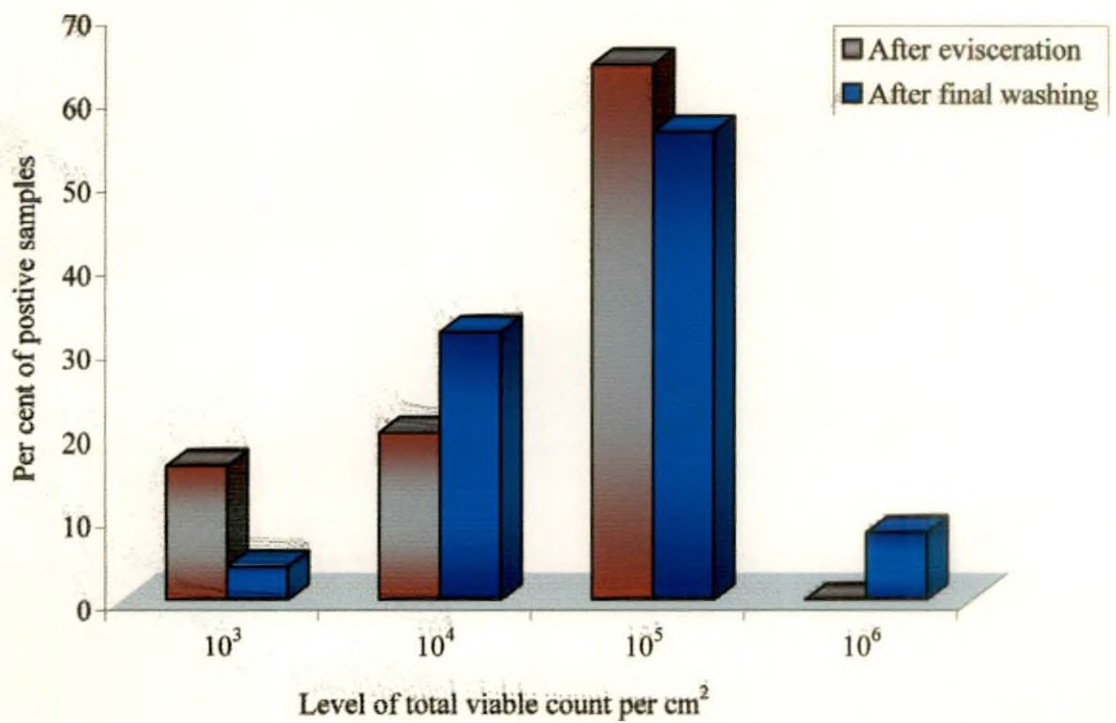
Samples		Level of TVC on carcasses (cfu/ $\text{cm}^2$ )			
No	Stage of dressing	$10^3$	$10^4$	$10^5$	$10^6$
25	AE	4 (16)	5 (20)	16 (64)	-
25	AFW	1 (4)	8 (32)	14 (56)	2 (8)

AE - After evisceration, AFW - After final washing

Figures in parenthesis indicate per cent

#### 4.1.2 Coliforms count

All samples collected from the carcasses after evisceration and after final washing were subjected to estimation of coliforms count (CC) and the mean count of both the group of carcasses is shown in table 4. Out of the 25 carcasses tested, only 15 (60%) samples collected after evisceration and 12 (48%) samples obtained



**Fig. 1. Distribution of pork carcasses based on TVC**

after final washing revealed the presence of the organism. However a high mean count was observed in the latter group of carcass.

**Table 4. Mean coliforms count of eviscerated and finally washed pork carcasses**

Samples		Mean $\pm$ SE (log <sub>10</sub> cfu/cm <sup>2</sup> )
No.	Stage of dressing	
25	AE	1.89 $\pm$ 0.17
25	AFW	1.99 $\pm$ 0.23

AE - After evisceration, AFW - After final washing

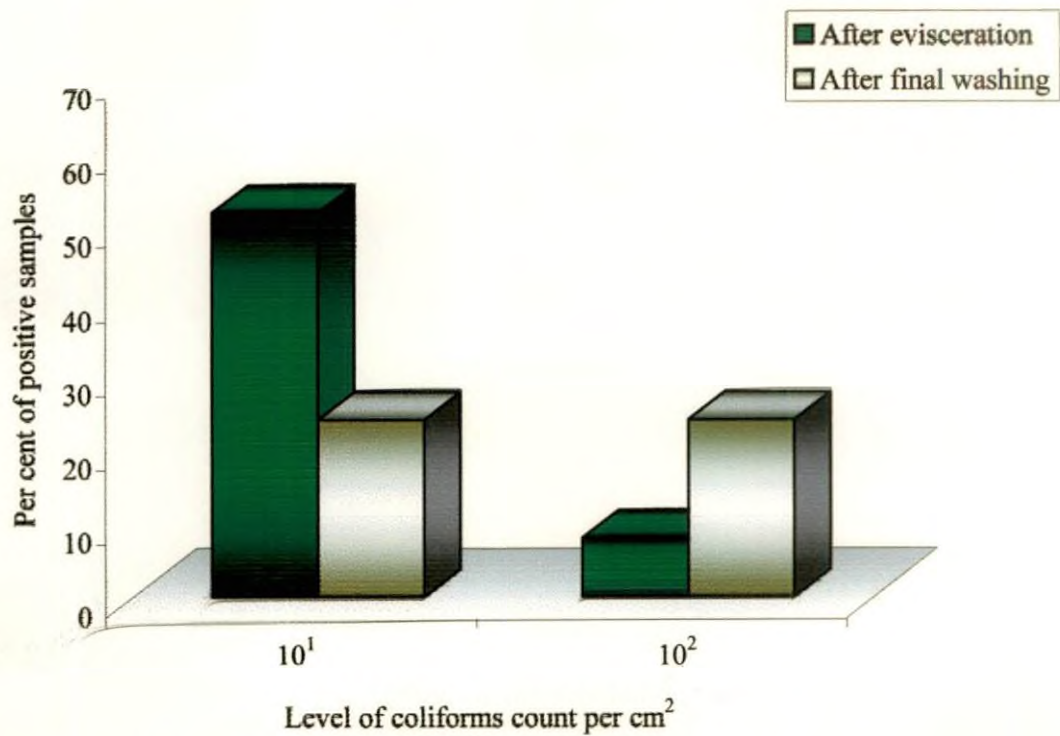
**4.1.2.1. Distribution of carcasses based on the level of coliforms count**

The distribution of both groups of carcasses based on coliforms count is shown in table 5 and Fig. 2. Out of the 25 carcass samples collected after evisceration, 40 per cent did not yield the organism. The organism was not detected in 52 per cent of samples obtained from finally washed carcass groups. However the carcasses from both the groups had count between 10<sup>1</sup> and 10<sup>2</sup> cfu/cm<sup>2</sup>. Fifty two percent of the samples collected from the carcasses after evisceration had the organism at the level of 10<sup>1</sup> cfu/cm<sup>2</sup>. Twenty four per cent each of the finally washed carcasses revealed count at the level of 10<sup>1</sup> and 10<sup>2</sup> cfu/cm<sup>2</sup>.

**Table 5. Distribution of carcasses based on the level of coliforms count**

Samples		Level of CC on carcasses (cfu/cm <sup>2</sup> )	
No	Stage of dressing	10 <sup>1</sup>	10 <sup>2</sup>
25	AE	13 (52)	2 (8)
25	AFW	6 (24)	6 (24)

AE - After evisceration, AFW - After final washing  
Figures in parenthesis indicate per cent



**Fig. 2. Distribution of pork carcasses based on CC**



#### 4.1.3 *Escherichia coli* count

The mean *Escherichia coli* count of the samples collected from the carcasses after evisceration and after final washing is given in table 6. *Escherichia coli* count of the samples collected after final washing had a higher mean count when compared to the samples obtained after evisceration.

**Table 6. Mean *Escherichia coli* count of eviscerated and finally washed pork carcasses**

Samples		Mean $\pm$ SE (log <sub>10</sub> cfu/cm <sup>2</sup> )
No	Stage of dressing	
25	AE	1.19 $\pm$ 0.08
25	AFW	1.29 $\pm$ 0.12

AE - After evisceration, AFW - After final washing

##### 4.1.3.1 *Distribution of carcasses based on the level of Escherichia coli count*

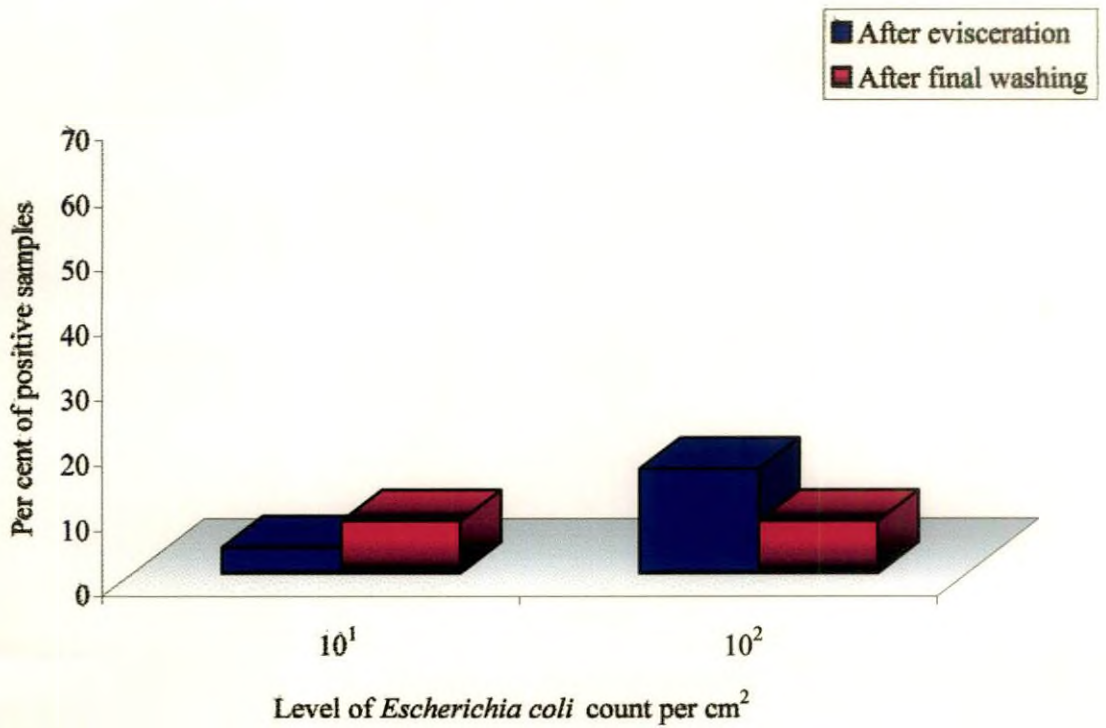
The distribution of carcasses based on *Escherichia coli* count is shown in table 7 and Fig. 3. *Escherichia coli* was not observed in 80 and 84 percent of carcasses after evisceration and after final washing, respectively. Both groups of carcasses had count at the level of 10<sup>1</sup> and 10<sup>2</sup> cfu/cm<sup>2</sup>. The count in 16 percent of samples collected after evisceration of carcasses had the count at the level of 10<sup>2</sup> cfu/cm<sup>2</sup> whereas, eight percent of the samples each from finally washed carcasses had count at the level of 10<sup>1</sup> and 10<sup>2</sup> cfu/cm<sup>2</sup>.

**Table 7. Distribution of carcasses based on the level of *Escherichia coli* count**

Samples		Level of ECC on carcasses (cfu/cm <sup>2</sup> )	
No.	Stage of dressing	10 <sup>1</sup>	10 <sup>2</sup>
25	AE	1 (4)	4 (16)
25	AFW	2 (8)	2 (8)

AE - After evisceration, AFW - After final washing

Figures in parenthesis indicate per cent



**Fig. 3. Distribution of pork carcasses based on ECC**

#### 4.1.4 Faecal streptococcal count

The mean faecal streptococcal count (FSC) of samples collected after evisceration and after final washing are depicted in table 8. The count of the samples taken after final wash of carcasses had a higher mean count as compared with those after evisceration.

**Table 8. Mean faecal streptococcal count of the eviscerated and finally washed pork carcasses**

Samples		Mean $\pm$ SE (log <sub>10</sub> cfu/cm <sup>2</sup> )
No.	Stage of dressing	
25	AE	3.19 $\pm$ 0.20
25	AFW	3.28 $\pm$ 0.20

AE - After evisceration, AFW - After final washing

##### 4.1.4.1 The distribution of carcasses based on the level of faecal streptococcal count

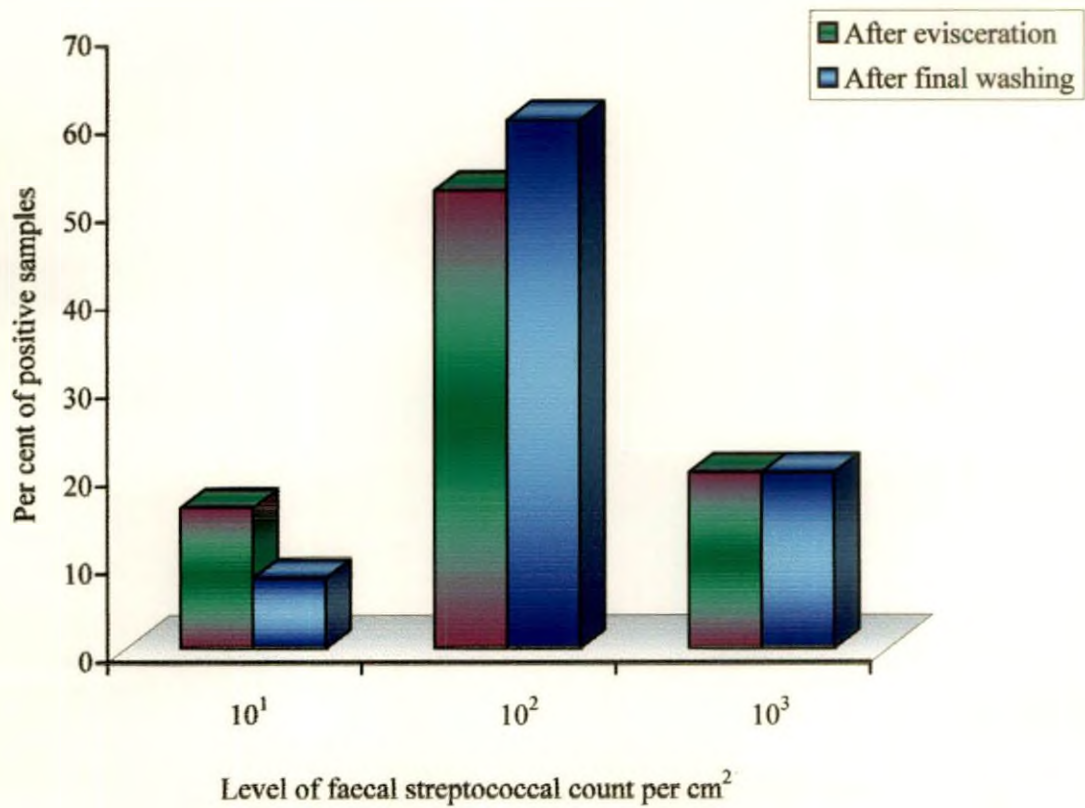
The distribution of pig carcasses after evisceration and after final washing based on faecal streptococcal count are given in table 9 and Fig. 4. Faecal streptococcal count was observed at the level of 10<sup>3</sup> cfu/cm<sup>2</sup> in both sets of carcasses and had the count in 20 per cent of samples. However the organism was not detected in 12 per cent of the samples in both groups. The highest percentage of carcasses in both the groups had count at the level of 10<sup>2</sup> cfu/cm<sup>2</sup>.

**Table 9. Distribution of carcasses based on faecal streptococcal count**

Samples		Level of FSC on carcasses (cfu/cm <sup>2</sup> )		
No.	Stage of dressing	10 <sup>1</sup>	10 <sup>2</sup>	10 <sup>3</sup>
25	AE	4 (16)	13 (52)	5 (20)
25	AFW	2 (8)	15 (60)	5 (20)

AE - After evisceration; AFW - After final washing

Figures in parenthesis indicate per cent



**Fig. 4. Distribution of pork carcasses based on FSC**

## 4.2 RELATIONSHIP BETWEEN THE BACTERIAL COUNT

### 4.2.1 Association between the mean bacterial count of carcass samples taken after evisceration

The correlation coefficient between mean bacterial count of samples from eviscerated carcasses is shown in table 10. Analysis of data revealed a positive and significant ( $P < 0.05$ ) association between CC and ECC. However, a non-significant but positive relationship was observed between mean TVC and ECC and also between ECC and FSC of eviscerated carcass samples. Negative and non-significant correlation was seen between TVC and CC, TVC and FSC and CC and FSC.

**Table 10. Correlation coefficient between mean bacterial count of eviscerated carcasses**

Bacterial Count	Correlation coefficient between mean bacterial count			
	TVC	CC	ECC	FSC
TVC		-0.070	0.130	-0.192
CC			0.514 *	-0.032
ECC				0.143

\* $P < 0.05$

TVC - total viable count, CC - coliforms count

ECC - *Escherichia coli* count, FSC - faecal streptococcal count

### 4.2.2 Association between the mean bacterial count of carcass samples taken after final washing

The relationship between the mean bacterial count of carcass samples taken after final washing is shown in table 11. The association between TVC and CC, TVC and ECC and CC and ECC was significant ( $P < 0.05$ ) but the former two associations showed a negative relationship. The relationship of FSC with all other

count was not significant. A negative correlation was observed between CC and FSC and FSC and ECC.

**Table 11. Correlation coefficient between mean bacterial count of finally washed carcasses**

Bacterial Count	Correlation coefficient between mean bacterial count			
	TVC	CC	ECC	FSC
TVC		-0.560*	-0.554*	0.231
CC			0.712*	-0.070
ECC				-0.052

\*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

All samples collected from eviscerated and finally washed carcasses were tested to isolate and identify *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*. The results are as follows:

##### 4.3.1 *Escherichia coli*

*Escherichia coli* was isolated from five (20%) eviscerated and four (16%) finally washed carcasses. All isolates, two each from every positive carcasses, were identified by cultural, morphological and biochemical tests. The 18 *Escherichia coli* were serotyped at the National *Escherichia* and *Salmonella* Centre, Central Research Institute, Kasauli, Himachal Pradesh. The serotypes isolated from each group of carcasses are shown in table 12. Of the 18 isolates, 15 fell into 12 serogroups and the remaining three were untypable. The serotypes O8, O11, O64, O88 and O101 were isolated solely from eviscerated carcasses while, serotypes O78, O119, O123 and O140 were isolated exclusively from finally washed

not use

carcasses. However, the serotypes O5, O25 and O60 were isolated from samples belonging to both groups of carcasses. Two isolates from eviscerated carcasses and one from finally washed carcasses were untypable.

**Table 12. Distribution of *Escherichia coli* serotypes isolated from eviscerated and finally washed carcasses**

Serotypes	Stage of dressing and number of isolates		Total
	AE	AFW	
O5	1	1	2
O8	1	-	1
O11	1	-	1
O25	1	1	2
O60	1	1	2
O64	1	-	1
O78	-	1	1
O88	1	-	1
O101	1	-	1
O119	-	1	1
O123	-	1	1
O140	-	1	1
UT	2	1	3

AE - After evisceration, AFW - After final washing

#### 4.3.2 *Staphylococcus aureus*

All samples collected from both eviscerated and finally washed carcasses revealed the presence of *Staphylococci*. However, *Staphylococcus aureus* was isolated from two (8%) of 25 eviscerated carcasses and from six (24%) of 25 finally washed carcasses.

### 4.3.3 *Salmonella*

All samples collected from both eviscerated and finally washed carcasses did not reveal the presence of salmonellae.

### 4.3.4 *Listeria monocytogenes*

None of the samples obtained from eviscerated and finally washed carcasses revealed the presence of the organism.

## 4.4 BACTERIAL COUNT OF ENVIRONMENTAL SAMPLES AND PROCESSING EQUIPMENTS

### 4.4.1 Air

The mean bacterial count of air samples obtained from slaughter hall, stunning and evisceration areas and chilling rooms of the pig slaughter line is given in table 13. Among the air samples from the four sites, the air at evisceration area had the highest count, while the lowest count was observed in the samples obtained from chilling room.

**Table 13. Mean bacterial count of air samples in the pig slaughter line**

Sampling area	Mean $\pm$ SE (cfu/min)
Slaughter hall	1.62 $\pm$ 0.83
Stunning area	2.01 $\pm$ 0.52
Evisceration area	2.08 $\pm$ 0.75
Chilling room	0.94 $\pm$ 0.19



#### 4.4.2 Water

The mean total viable count (TVC), coliforms count (CC), and *Escherichia coli* count (ECC) of water used in the pig slaughter line are shown in table 14. Pond water samples had the highest mean total viable count. However, *Escherichia coli* count was highest in hand washings of personnel. Coliforms count was seen highest in scalding tank water and the count was found at the lowest level in tap water. None of the pond water and tap water samples revealed *Escherichia coli*.

**Table 14. Mean bacterial count of water used in the pig slaughter line**

Source	Bacterial count (log <sub>10</sub> cfu/ml)		
	TVC	CC	ECC
Pond water	5.10 ± 0.79	1.39 ± 0.77	-
Tap water	2.89 ± 0.12	0.45 ± 0.15	-
Scalding tank water	4.28 ± 0.78	1.59 ± 0.76	1.12 ± 0.75
Hand wash of personnel	4.96 ± 0.82	1.26 ± 0.78	1.18 ± 0.77

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

#### 4.4.3 Processing equipments

The mean bacterial count on the bell scraper, knife, steel, cutting board, cutting table, carcass splitter and HDPE pouches are shown in table 15. Samples collected from bell scraper had the highest mean total viable count and the count was least in HDPE pouches. None of the samples revealed the presence of *Escherichia coli*. Samples obtained from knives, cutting board and cutting table had faecal streptococci, with the highest level of organism observed in cutting board samples. Coliforms were not detected in the samples obtained from bell scraper and HDPE pouches. The organism was observed in all other samples and the highest count was seen in samples collected from carcass splitter.

**Table 15. Mean bacterial count of processing equipments used in the pig slaughter line**

Equipments	Bacterial count (log <sub>10</sub> cfu/cm <sup>2</sup> or per ml)			
	TVC	CC	ECC	FSC
Bell scraper	5.93 ± 0.80	-	-	-
Knife	2.36 ± 0.76	1.11 ± 0.73	-	1.13 ± 0.70
Steel	3.08 ± 0.75	1.57 ± 0.74	-	-
Cutting board	4.94 ± 0.87	1.24 ± 0.94	-	1.38 ± 0.86
Cutting table	4.28 ± 0.87	1.26 ± 0.83	-	1.29 ± 0.79
Carcass splitter	4.96 ± 0.16	2.24 ± 0.13	-	-
HDPE pouches	1.18 ± 0.76	-	-	-

TVC = Total viable count, CC = Coliforms count

ECC = *Escherichia coli* count, FSC = Faecal streptococcal count

# *Discussion*

## 5. DISCUSSION

During the present investigation, pork carcass samples were collected after evisceration and after final washing in the processing line of a meat processing plant, in order to evaluate the hygienic status of carcasses produced in the plant. All samples were tested to detect the level of bacterial load per  $\text{cm}^2$  on the carcass surface and also to detect the presence of certain bacteria of public health significance. Studies were also made to assess the role of environment, personnel and equipments on the bacterial contamination of carcasses.

### 5.1 BACTERIAL COUNT

In the study, an area of  $100 \text{ cm}^2$  each, was swabbed from five different sites on the carcasses viz., jowl, shoulder, bacon, loin and ham, after evisceration and after final washing. The samples collected from each of the evisceration or finally washed carcass were pooled so as to form a uniform representative sample. The bacterial load on the carcasses was determined by estimating the total viable count, coliforms count, faecal streptococcal count and *Escherichia coli* count.

#### 5.1.1 Total viable count

Analysis of variance test revealed that the mean total viable count of carcasses after final washing was significantly ( $P < 0.05$ ) higher than that of eviscerated carcasses. The mean total viable count of eviscerated carcasses was two log greater than that reported by Rahkio and Korkeala (1992) and Rivas *et al.* (2000). The mean count of finally washed carcass samples was four logs higher than that reported by Yu *et al.* (1999) and about two log greater than that recorded by Bolton *et al.* (2002). In the eviscerated carcasses, 64 per cent had count at the level of  $10^5 \text{ cfu/cm}^2$  and 56 per cent of finally washed carcass had the count at the above level. The difference between the high count of eviscerated and finally washed carcasses was one log. The increased count in finally washed carcasses could be attributed to close contact between carcasses, contamination from splitter, splashing of water during power hosing and extensive handling of carcasses by personnel.

Total viable count is used as an index of sanitary quality and handling history of foods (Jay, 1978). The high count in freshly dressed carcasses is an indication of the poor hygienic practices followed during dressing and the count may shorten the keeping quality of such carcasses.

### 5.1.2 Coliforms count

The finally washed carcasses had a high mean coliforms count,  $1.99 \pm 0.23 \log_{10} \text{ cfu/cm}^2$ , as compared to the count of the eviscerated carcasses,  $1.89 \pm 0.17 \log_{10} \text{ cfu/cm}^2$ . The latter count was  $0.89 \log_{10} \text{ cfu/cm}^2$  higher than that reported by Rivas *et al.* (2000). However, the count on finally washed carcasses was one log greater than that recorded by Yu *et al.* (1999). Coliforms count was observed at the level of  $10^2 \text{ cfu/cm}^2$  in both groups of carcasses. The count at the above level was observed in eight percent of the former group of carcass and 24 per cent of the latter group of carcasses. Coliforms count is used to assess the over all quality and hygienic condition prevailing during processing of food (Kornacki and Johnson, 2001). The high level of the count indicates carcass contamination might be of faecal origin or from water contaminated with the organisms.

### 5.1.3 *Escherichia coli* count

The mean *Escherichia coli* count of the eviscerated carcasses was  $1.19 \pm 0.08 \log_{10} \text{ cfu/cm}^2$ , which is almost similar to that recorded by Rivas *et al.* (2000). The count in finally washed carcasses was  $1.29 \pm 0.12 \log_{10} \text{ cfu/cm}^2$ , which is similar to that reported by Gill *et al.* (2000a). The organism was not observed in 80 per cent of eviscerated and in 84 per cent of the finally washed carcasses. The organism was observed in 16 per cent of eviscerated and eight per cent of finally washed carcasses at the level of  $10^2 \text{ cfu/cm}^2$ . Only five (20%) out of 25 eviscerated carcasses and four (16%) out of the 25 finally washed carcasses had the organism and the count in these samples were much higher than that recommended by USDA (Gill,2000). Though the organism is of intestinal origin, at times, it is absent in the intestinal tract of hogs (Jay, 1996). The contamination of carcasses might have occurred from intestinal contents, contaminated water and also from the personnel engaged in various dressing processes.

#### 5.1.4 Faecal streptococcal count

The mean faecal streptococcal count of the eviscerated and finally washed carcasses was  $3.19 \pm 0.20 \log_{10} \text{ cfu/cm}^2$  and  $3.28 \pm 0.20 \log_{10} \text{ cfu/cm}^2$ , respectively. In both groups, the count was observed at the level of  $10^3 \text{ cfu/cm}^2$ . The organism is normally present in mammalian faeces and act as indicators of poor factory sanitation (Brown and Baird-Parker, 1982). The organism is widely distributed in slaughterhouses and are occasionally associated with bone taint or sliminess in carcasses (Jay, 1978). The presence of the organism in 88 per cent of the samples indicate poor hygienic practices at different levels of dressing of carcasses.

### 5.2 RELATIONSHIP BETWEEN BACTERIAL COUNT

#### 5.2.1 After evisceration

Analysis of data revealed a positive and significant ( $P < 0.05$ ) relation between CC and ECC, which is in accordance with the reports of Rivas *et al.* (2000). It was observed that a negative and non-significant association existed between TVC and CC and TVC and FSC. A similar association was found between CC and FSC. However the relationship between TVC and ECC and ECC and FSC were non-significant, but positive. From the observation of the study, it may be inferred that the contamination of carcasses with coliforms might be of intestinal origin since the only positive and significant association was observed between CC and ECC.

#### 5.2.2 After final washing

A positive and significant ( $P < 0.05$ ) relationship was observed between CC and ECC. This indicates that carcass contamination was mainly of intestinal origin. However, TVC and CC and also TVC and ECC had a negative but significant ( $P < 0.05$ ) association. Such a relationship indicates that as TVC increases, both CC and ECC decreases. This may be attributed to the reduction of bacteria during washing. The increase in ECC with CC might be due to the redistribution of organisms during washing the internal body cavities, splashing of water between carcasses during power hosing and the subsequent surface contact and due to the extensive handling by personnel.

### 5.3 ISOLATION OF BACTERIA

Samples collected from the eviscerated and finally washed carcasses were examined to detect the presence of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*.

#### 5.3.1 *Escherichia coli*

*Escherichia coli* was isolated from 20 per cent of eviscerated and 16 per cent of finally washed carcasses. A total of 18 *Escherichia coli* were recovered from the carcasses. On serotyping, 15 isolates fell into 12 serogroups viz., O5, O8, O11, O25, O60, O64, O78, O88, O101, O119, O123 and O140 and three isolates, were untypable.

The serotypes O8, O11, O25 and O101 belonged to the enterotoxigenic *Escherichia coli* (ETEC) group. These serotype resemble *Vibrio cholerae* with their enterotoxins namely heat labile (LT) and heat stable (ST) toxins. These are responsible for fatal infantile and traveller's diarrhoea in man. Food handlers and contaminated water supply have been implicated as the cause. The isolation of serotype O11 from pork samples has been reported by Nair *et al.* (1984), Borah *et al.* (1988b) and Sulthienkul *et al.* (1990). The strain causes parenteral septic infection in man.

Enterohaemorrhagic *Escherichia coli* (EHEC) produces one or two Shiga-like toxins. The serotypes O5 and O88 belong to this group. These produce haemolytic uremic syndrome (HUS) and haemorrhagic colitis (HC) in man. Foods contaminated with these serotypes have been associated with several outbreaks. Samadpour *et al.* (1994) reported the isolation of serotype O88 from pork samples.

Serotype O78 belongs to enteroaggregative *Escherichia coli* (EAEC) and is responsible for persistent diarrhoea in children.

The serotype O119 belongs to enteropathogenic (EPEC) group of *Escherichia coli*. Though the serotype does not produce enterotoxins, it causes diarrhoea in man.

The serotype O5 belongs to the verocell cytotoxin producing group of *Escherichia coli*. Isolation of VTEC organism has been reported by Bouvet *et al.* (2002b). Pigs may be a vehicle for pathogenic VTEC strains associated with post-weaning diarrhoea and oedema disease in weaned pigs (Smith and Cheasty, 1998). It is one of the most important, recently emerged group of pathogen, in food chain, transmitted to human through direct or indirect contamination of foods including pork, by faecal material.

The presence of pathogenic strains of *Escherichia coli* in the pork carcasses during the study, indicate the potential risk of infection to man. Presence of the organism in finally washed carcasses indicates that the organism has survived the pathogen reduction steps in the dressing process, which indicates the cross contamination and unhygienic practices during dressing.

### 5.3.2 *Staphylococcus aureus*

Staphylococci were present in all samples of eviscerated and finally washed carcasses. However *Staphylococcus aureus* was detected from two (8%) out of 25 eviscerated and six (24%) out of 25 finally washed carcasses. Isolation of the organism has also been reported by Nair *et al.* (1984), Saide-Albornoz *et al.* (1995) and Rho *et al.* (2001).

Staphylococci are ubiquitous in nature and considered as opportunistic pathogens. *Staphylococcus aureus* constitutes a normal part of the microflora of animal and human body, being found on skin and hair, nose, mouth and throat (Martin and Myers, 1994). Any food that provides a good medium for the growth of the organism may be involved in food poisoning. Foods commonly associated with staphylococcal food poisoning are meat (beef, pork, poultry), salads and dairy products. Contamination of dressed pig carcasses by the organism is common and often unavoidable. Foods subjected to post process contamination with *Staphylococcus aureus* pose a significant hazard due to elimination of competitive organisms that normally restrict the growth of *Staphylococcus aureus* and the production of enterotoxins. Contamination of food occur mainly by process line workers with hand or arm lesions caused by the organism, coming in contact with



the food. So also, contaminated processing surfaces is an important source of the organism (Bergdoll, 1990; Lancette and Bennett, 2001). In the present study, the presence of the organism in both groups of carcasses indicates poor hygienic practices followed during production. The presence of increased load of *Staphylococcus aureus* in finally washed carcasses than the eviscerated group indicates contamination by extensive handling of the carcasses by meat handlers in the processing line.

### 5.3.3 *Salmonella*

None of the samples from both eviscerated and finally washed carcasses revealed the presence of *Salmonella*. Similar reports have been recorded by Gill and Bryant (1993), Miller *et al.* (1997), Carr *et al.* (1998), Rho *et al.* (2001) and Thorberg and Engvall (2001). However, isolation of the organism from pork carcasses were reported by Mafu *et al.* (1989), Saide-Albornoz *et al.* (1995), Berends *et al.* (1997), Korsak *et al.* (1998), Giovannacci *et al.* (2001), Swanenburg *et al.* (2001) and Bolton *et al.* (2002).

### 5.3.4 *Listeria monocytogenes*

All samples from eviscerated and finally washed carcasses did not yield *Listeria monocytogenes*. The results corroborates with the findings of Nesbakken *et al.* (1994), Miller *et al.* (1997), and Rho *et al.* (2001). However, Adesiyun and Krishnan (1995), Saide-Albornoz *et al.* (1995), Takeshige *et al.* (1995), Korsak *et al.* (1998) and Autio *et al.* (2000) have reported the isolation of the organism from pork carcasses.

## 5.4 ENVIRONMENT AND PROCESSING EQUIPMENTS

The mean bacterial count of air samples at the area of slaughter hall, stunning, evisceration and chilling room in the pork processing line were  $1.62 \pm 0.83$ ,  $2.01 \pm 0.52$ ,  $2.08 \pm 0.75$  and  $0.94 \pm 0.19$  cfu/min, respectively. The result is 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) in slaughterhouses.

The study revealed the contribution of airborne bacteria in the contamination of pork carcasses during the various stages of dressing.

The mean TVC of water samples from pond, tap, scalding tank and hand washings of personnel were  $5.10 \pm 0.79$ ,  $2.89 \pm 0.12$ ,  $4.28 \pm 0.78$  and  $4.96 \pm 0.82$   $\log_{10}$  cfu/ml, respectively. The corresponding coliforms count were  $1.39 \pm 0.77$ ,  $0.45 \pm 0.15$ ,  $1.59 \pm 0.76$  and  $1.26 \pm 0.78$   $\log_{10}$  cfu/ml. *Escherichia coli* count in scalding tank water and hand washings of personnel were  $1.12 \pm 0.75$  and  $1.18 \pm 0.77$   $\log_{10}$  cfu/ml. The total viable count of the water samples is similar to that obtained by Tarwate *et al.* (1993), whereas, the coliforms count was four to six log higher than the results of the present study. Water used in abattoir should be potable (Gracey *et al.* 1999). The findings show that the water used in the abattoir was far from international drinking water quality standards prescribed by W.H.O. (Rajvaidya and Markandey, 1992). The higher count in the water samples indicate its unwholesomeness and its role in contamination of carcasses especially during washing.

The TVC of various processing equipments ranged between  $2.36 \pm 0.76$  and  $5.93 \pm 0.80$   $\log$  cfu/cm<sup>2</sup> or per ml. The CC varied between  $1.11 \pm 0.73$  and  $2.24 \pm 0.13$   $\log$  cfu/cm<sup>2</sup> or per ml and the FSC ranged between  $1.13 \pm 0.70$  and  $1.38 \pm 0.86$   $\log$  cfu/cm<sup>2</sup> or per ml. Knife and steel samples revealed TVC at the level of  $2.36 \pm 0.76$  and  $3.08 \pm 0.75$  cfu per ml, respectively. The result is lower than that reported by Rivas *et al.* (2000). Similarly coliforms count of the samples were varied between  $1.11 \pm 0.73$  and  $1.57 \pm 0.74$  cfu/ml which is one log higher than that of the above report. Samples from cutting table revealed a mean CC of  $1.26 \pm 0.83$  cfu/cm<sup>2</sup> which is lower than that reported by Karr *et al.* (1996).

The findings of the bacterial quality of environment and processing equipment samples indicate their significance in the contamination of pork carcasses during its production.

# *Summary*

## 6. SUMMARY

In the present investigation, 25 pork carcasses were randomly selected from the dressing line of a meat processing plant located at Kochi, in Kerala. From each carcass, 500 cm<sup>2</sup> area was swabbed from one half of the carcass, immediately after evisceration, which consisted of 100 cm<sup>2</sup> each from jowl, shoulder, bacon, loin and ham. These swab samples were pooled into 500 ml diluent, so as to form, the initial test sample, which was used for assessing the bacteriological quality of carcasses by estimating total viable count, coliforms count, *Escherichia coli* count and faecal streptococcal count. The sample was also used to isolate and identify *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*. After final washing of same carcasses, swab samples were collected as described above, processed and bacteriological evaluations were made as described for the other half of the carcass. The bacteriological quality of samples from environment, processing equipments, hand washings of personnel, packaging material and scalding tank water were also evaluated to determine the level of various bacterial load. All bacterial count of carcass samples were subjected to statistical analysis.

Analysis of variance test of the data revealed that the mean TVC on finally washed carcasses was significantly ( $P < 0.05$ ) higher than the eviscerated carcasses. The former group of carcasses had a high mean count ( $5.31 \pm 0.16 \log_{10} \text{ cfu/cm}^2$ ). The count of the finally washed carcasses varied between  $10^3$  and  $10^6 \text{ cfu/cm}^2$ . The count in 56 per cent samples was at the level of  $10^5 \text{ cfu/cm}^2$  and 32 per cent had the count at the level of  $10^4 \text{ cfu/cm}^2$ . In eight per cent of the carcasses, the count was at the level of  $10^6$ , four per cent had the count at the level of  $10^3 \text{ cfu/cm}^2$ . In the eviscerated carcasses, 64 per cent had count at the level of  $10^5 \text{ cfu/cm}^2$ . The count in 20 and 16 per cent of the carcasses was at the level of  $10^4$  and  $10^3 \text{ cfu/cm}^2$ , respectively.

The mean coliforms count of the eviscerated and finally washed carcasses was  $1.89 \pm 0.17$  and  $1.99 \pm 0.23 \log_{10} \text{ cfu/cm}^2$ , respectively. The count in both groups of carcasses varied from  $10^1$  to  $10^2 \text{ cfu/cm}^2$ . However, 40 per cent carcasses of the former group and 52 per cent carcasses in the latter group did not reveal the

presence of the organism. In eight per cent of eviscerated carcasses the count was at the level of  $10^2$  cfu/cm<sup>2</sup> and 52 per cent of the carcasses had count at the level of  $10^1$  cfu/cm<sup>2</sup>. The count in 24 per cent of finally washed carcass was at the level of  $10^1$  cfu/cm<sup>2</sup> and same per cent had count at the level of  $10^2$  cfu/cm<sup>2</sup>.

The mean *Escherichia coli* count in the eviscerated and finally washed carcasses was  $1.19 \pm 0.08$  and  $1.29 \pm 0.12$  log<sub>10</sub> cfu/cm<sup>2</sup>, respectively. The organism was not detected in 80 per cent of former group and 84 per cent of latter group of carcasses. In eviscerated carcasses, 16 per cent had count at the level of  $10^2$  cfu/cm<sup>2</sup> and in four per cent, the count was at the level of  $10^1$  cfu/cm<sup>2</sup>. In the finally washed carcasses, eight per cent each had count at the level of  $10^1$  and  $10^2$  cfu/cm<sup>2</sup>, respectively.

The mean faecal streptococcal count in eviscerated carcasses samples was  $3.19 \pm 0.20$  log<sub>10</sub> cfu/cm<sup>2</sup> and in finally washed carcass samples was  $3.28 \pm 0.20$  log<sub>1</sub> cfu/cm<sup>2</sup>. The organism was not detected in 12 per cent of carcasses from both the groups. The count in 20 per cent carcasses of both the groups was at the level of  $10^3$  cfu/cm<sup>2</sup>. In the eviscerated carcasses, 52 per cent and 16 per cent had count at the level of  $10^2$  and  $10^1$  cfu/cm<sup>2</sup>, respectively. However, the count in 60 per cent and eight per cent of the finally washed carcasses was at the level of  $10^2$  and  $10^1$  cfu/cm<sup>2</sup>, respectively.

The data were subjected to correlation coefficient test. A positive and significant ( $P < 0.05$ ) correlation was observed between the mean CC and ECC of eviscerated carcasses. However, a non-significant but positive correlation was observed between TVC and ECC and ECC and FSC. A negative and non-significant correlation was seen between TVC and CC, TVC and FSC and CC and FSC. Finally washed carcasses showed a positive and significant ( $P < 0.05$ ) correlation between CC and ECC. However, the correlation between TVC and CC and TVC and ECC was negative but significant ( $P < 0.05$ ).

*Escherichia coli* was isolated from five (20 per cent) eviscerated and four (16 per cent) finally washed carcasses. The 18 isolates recovered from both groups of carcasses were serotyped at the Central Research Institute, Kasauli, Himachal

Pradesh. Of the isolates, 12 were serotyped as O5, O8, O11, O25, O60, O64, O78, O88, O101, O119, O123 and O140 and three were untypable. The isolates from eviscerated carcasses included O5, O8, O11, O25, O60, O64, O88 and O101 and those from finally washed carcasses included O5, O25, O60, O78, O119, O123 and O140. The isolates from both groups of carcasses belonged to enterotoxigenic *Escherichia coli* (O8, O11, O25 and O101), enterohaemorrhagic *Escherichia coli* (O5 and O88), enteroaggregative *Escherichia coli*. (O87), enteropathogenic *Escherichia coli* and vero cell cytotoxic *Escherichia coli* (O5). All carcass samples, after evisceration and final washing, revealed the presence of staphylococci. However, *Staphylococcus aureus* was isolated from two (eight per cent) of the eviscerated carcasses and six (24 per cent) of finally washed carcasses.

All carcass samples belonging to both the groups did not reveal the presence of *Salmonella*.

None of the samples from both the groups of carcasses revealed the presence of *Listeria monocytogenes*.

Air samples at the evisceration site had the highest mean bacterial count ( $2.08 \pm 0.75$  cfu/min), while the lowest mean count was observed in samples from chilling room ( $0.94 \pm 0.19$  cfu/min.). Pond water samples had the highest mean total viable count ( $5.10 \pm 0.79 \log_{10}$  cfu/ml). The highest level of coliforms count was seen in scalding tank water samples ( $4.28 \pm 0.78 \log_{10}$  cfu/ml). The highest *Escherichia coli* count was observed in hand washings of personnel. None of the pond and tap water samples revealed the presence of the organism. Among the samples collected from processing, bell scraper samples had the highest mean total viable count ( $5.93 \pm 0.80 \log_{10}$  cfu/ml) and the least count was noticed in HDPE pouches ( $1.18 \pm 0.76 \log_{10}$  cfu/ml). Samples from carcass splitter had the highest coliforms count ( $2.24 \pm 0.13 \log_{10}$  cfu/ml) and the highest mean faecal streptococcal count was seen in meat cutting board samples ( $1.38 \pm 0.86 \log_{10}$  cfu/cm<sup>2</sup>). None of the samples from processing equipments revealed the presence of *Escherichia coli*.

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# **EVALUATION OF BACTERIOLOGICAL QUALITY OF PORK CARCASSES IN A PROCESSING PLANT**

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**Abstract of the thesis submitted in partial fulfilment of the  
requirement for the degree of**

## **Master of Veterinary Science**

**Faculty of Veterinary and Animal Sciences  
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**2003**

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## ABSTRACT

During the present study, 25 pork carcasses were randomly selected from the dressing line of a pork processing plant, in order to evaluate the hygienic status of the carcasses produced in the plant. From each carcass, after evisceration and also after final washing, 500 cm<sup>2</sup> area was swabbed, which consisted of 100 cm<sup>2</sup> each from jowl, shoulder, bacon, loin and ham areas. Samples collected, after evisceration were pooled into 500 ml diluent, which formed the initial test samples. Similarly, the initial test sample was also prepared with the samples collected from the finally washed carcasses. This sample was tested to evaluate the bacterial quality of carcasses by estimating total viable count, coliforms count, *Escherichia coli* count and faecal streptococcal count. The sample was also used for the isolation and identification *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*. Samples from environment, processing equipment, hand wash of personnel, scalding tank water and HDPE pouches were also tested to estimate the bacterial load. Analysis of variance test revealed significant ( $P < 0.05$ ) difference between the mean total viable count of the eviscerated and finally washed carcasses. The mean count in the former and latter groups of carcasses was  $5.14 \pm 0.16$  and  $5.31 \pm 0.16$ ,  $\log_{10}$  cfu/cm<sup>2</sup>, respectively. In 64, 20 and 16 per cent of the eviscerated carcasses the count was at levels  $10^5$ ,  $10^4$  and  $10^3$  cfu/cm<sup>2</sup>, respectively. In the latter group of carcasses, eight, 56, 32 and four per cent had the count at the level of  $10^6$ ,  $10^5$ ,  $10^4$  and  $10^3$  cfu/cm<sup>2</sup>, respectively.

The mean coliforms count of eviscerated carcasses was  $1.89 \pm 0.17 \log_{10}$  cfu/cm<sup>2</sup> and the count in the finally washed carcasses was  $1.99 \pm 0.23 \log_{10}$  cfu/cm<sup>2</sup>. Eight and 24 per cent of samples belonging to both groups had the count at the level of  $10^2$  cfu/cm<sup>2</sup>. The count in 52 and 24 per cent of eviscerated and finally washed carcass samples was at the level of  $10^1$  cfu/cm<sup>2</sup>.

Eviscerated carcasses had a mean *Escherichia coli* count of  $1.19 \pm 0.08 \log_{10}$  cfu/cm<sup>2</sup> and the count in finally washed carcasses was  $1.29 \pm 0.12 \log_{10}$  cfu/cm<sup>2</sup>. The organism was not detected in 80 and 84 per cent of eviscerated and finally washed carcasses, respectively. The count in 16 and four per cent of the former group of carcasses was at levels  $10^2$  and  $10^1$  cfu/cm<sup>2</sup>, respectively. In eight per cent each of finally washed carcasses, the count was at the level of  $10^2$  and  $10^1$  cfu/cm<sup>2</sup>.

The eviscerated and finally washed carcasses had a mean faecal streptococcal count of  $3.19 \pm 0.20$  and  $3.28 \pm 0.20 \log_{10}$  cfu/cm<sup>2</sup>, respectively. The count on 12 per cent of carcasses in each of the above groups, did not reveal the organism. However, in both the groups of carcasses, 20 per cent of samples each had the count at  $10^3$  cfu/cm<sup>2</sup>. The count in 52 per cent of the eviscerated and 60 per cent of the finally washed carcasses was at the level of  $10^2$  cfu/cm<sup>2</sup>. The count at the level of  $10^1$  cfu/cm<sup>2</sup> was observed in 16 per cent of the eviscerated and eight per cent of the finally washed carcasses.

Analysis of the data revealed positive and significant ( $P < 0.05$ ) association between CC and ECC in eviscerated and finally washed carcasses. However, a negative but significant ( $P < 0.05$ ) correlation was observed between TVC and CC and TVC and ECC in the finally washed carcasses.

*Escherichia coli* was isolated from 20 per cent of eviscerated carcasses and 16 per cent of finally washed carcasses. Out of 18 isolates recovered from both the groups, 15 fell into 12 serotypes and three were untypable. The serotypes include O5, O8, O11, O25, O60, O64, O78, O88, O101, O119, O123 and O140.

*Staphylococcus aureus* was isolated from eight and 24 per cent of samples belonging to eviscerated and finally washed carcasses, respectively.

None of the carcass samples in both the groups revealed the presence of *Salmonella* and *Listeria monocytogenes*.

Air samples at the evisceration area had the highest bacterial count and the lowest was observed at the chilling room. Pond water samples had the highest mean total viable count and coliforms population was seen highest in scalding tank water. *Escherichia coli* count was highest in hand washing of personnel. None of the pond and tank water samples revealed presence of the organism. Samples collected from the bell scraper had the highest mean total viable count and least in packaging material. Samples obtained from carcass splitter had the highest coliforms count. None of the processing equipments revealed the presence of *Escherichia coli*. Cutting board samples showed the highest faecal streptococcal count.