AN EVALUATION OF THE MICROBIAL CONTAMINATION OF CARCASSES AT A LOCAL RED MEAT TYPE C ABATTOIR

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CONTENTS

Opsomming

Die higiëniese praktyke by `n plaaslike abattoir is gemonitor. Die belangrikste mikrobiese organismes wat bederf in rooivleis versaak, is vanaf tien beeskarkasse verkry en gekarakteriseer. In plaas daarvan om slegs die finale produk te ëvalueer, is monsters met gereelde intervalle (vier) langs die bewerkingslyn, van net nadat die vel verwyder is, tot na `n verkoelingstydperk van tussen sestien en agtien ure, geneem.

Totale aërobiese plaattellings, Gram-negatiewe tellings en melksuur bakteriëse tellings is bepaal. Die hoogste mikrobiese tellings (ongeveer 3 $\log_{10}$ getalle per cm^2) is konstant vanaf die totale aerobiese plaattellings verkry, gevolg deur die Gram-negatiewe tellings (ongeveer 2 $\log_{10}$ getalle per cm^2), terwyl daar geen groei op die melksuur agar waargeneem is nie.

Die karakterisering van die 104 predominant bakteriese-isolate vanaf die totale aërobiese plaattellings van die karkasse, het aangetoon dat Pseudomonas spp. (26%), Aeromonas spp. (13%), Acinetobacter en Micrococcaceae (10% elk) en `n kleiner hoeveelheid van die spesies Moraxella (8%), Alteromonas (5%), Brochothrix en Corynebacterium (4% elk) en Enterobacteriaceae en Staphylococcus (3% elk) teenwordig was.

Die mikroflora van die Gram-negatiewe tellings van die karkasse is ook deur die Pseudomonas spp. (50%) gedomineer, terwyl Enterobacteriaceae (15%), Aeromonas spp. (10%), die Moraxella-groep (5%) en nie-kweekbare bakterieë (20%) ook voorgekom het.

Die moontlike invloed van die bewerkingsproses en die verkoelingsproses van die karkas op die bakteriese tellings is bepaal. Ten spyte van die duidelik waarneembare tendens, waar die bakteriese getalle afneem na die verkoelingsproses, het hierdie waarneming geen statistiese beduidenheid nie.

Abstract

The hygiene practices at a local abattoir were monitored. The main microbial spoilage organisms associated with red meat carcasses were enumerated and characterized from ten bovine carcasses. Instead of evaluating the final product only, samples were taken at regular positions (four) along the carcass processing/dressing line from just after skin removal (flaying) until after a chilling period of between eighteen and twenty four hours.

Total aerobic plate counts, Gram-negative counts and lactic acid bacterial counts were determined. The highest microbial numbers were consistently obtained from the total aerobic plate counts (approximately 3 $\log_{10}$ numbers per cm^2) followed by the Gram negative counts (approximately 2 $\log_{10}$ numbers per cm^2) and there were no growths observed on the lactic acid bacteria agar.

Characterization of the 104 predominant bacterial isolates from total aerobic plate counts of the carcasses indicated the presence of *Pseudomonas* spp.(25%), *Aeromonas* spp (13%), *Acinetobacter* and Micrococcaceae (10% each) and fewer amounts of species of *Moraxella*(8%), *Alteromonas* (5%), *Brochothrix* and *Corynebacterium* (4% each), Enterobacteriaceae and *Staphylococcus* (3% each).

The microflora from the Gram-negative counts of the carcasses was also dominated by the *Pseudomonas* spp. (50%), Enterobacteriaceae (15%), *Aeromonas* spp. (10%) the *Moraxella* group (5%) and non-reculturable (20%).

The possible effects of the carcass dressing procedures and the chilling process on the bacterial counts were determined. Despite the apparent observable trends where

the bacterial numbers seemed to decrease after the chilling process they were of no statistical significance.

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Chapter 1

Introduction

Microbial quality control does not entail microbial evaluation of the finished product only. Current microbial evaluation processes emphasize testing for several critical processing stages. The tendency is for analysts to evaluate critical control points along the processing line. This is contained in the hazard analysis critical control process (HACCP). No sampling information data are available for evaluation of the microbiological status of carcasses at any red meat abattoir in the North West province and as such the project was to assess this situation at a commercial red meat abattoir in the North West Province.

Sampling data were collected from three sampling sites along a carcass dressing line on fresh carcasses immediately after slaughter and a final, fourth reading was done on the carcasses after a chilling process of 18-24 hours. The samples were then diluted and cultured on nutrient agar medium, desoxycholate citrate agar and MRS agar. All ten readings were treated as replicates and averaged out. There seemed to be an increase in the bacterial counts from after skin removal (position 1) till after the wash (position 3) in the fore-rib on both nutrient agar and on desoxycholate citrate agar. There was observed a sharp increase in the number of bacteria recovered from the neck just after evisceration. There also was observed a decrease in the microbial counts after the chilling process. The data were then analysed using the *Statistica* computer program to test for statistical significance of the apparent trends in increases or decreases in the numbers of the bacteria along the carcass processing line.

Predominant bacterial isolates or colony forming units were picked up and sub-cultured on agar slants. The predominant isolates were subjected to some tests and were characterised to genus or family level. The main genera picked up were the *Pseudomonas* spp., *Aeromonas* spp, Micrococcaceae and the *Moraxella/Acinetobacter* group amongst the Gram negative and *Brochothrix* spp. which are Gram positive. Relative ratios of the isolates were then compared to literature ratios. Possible biochemical effects of the isolates on meat were discussed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

What is meat? Generally, it is considered to be the edible flesh, including fat, and the skin, rind, gristle and sinew of animals or birds which are normally acceptable for consumption by man (Laurie, 1975; Hanson, 1975). From a tradesman's point of view it is the carcass of beef, sheep, goat, pig and poultry and certain edible glands and organs. From an anatomist's view-point it is muscle, bone, cartilage and fat. From a biochemist's point of view it is protein, lipid, moisture and ash. From a consumer's point of view 'meat' refers to red meat (that of cattle, pig, and sheep) but may include the meat of poultry (chicken, turkey, duck, and geese) and is looked upon as food - one of the most valuable in monetary terms, in nutritive content and in satiety value (Naumann, 1984; Briggs *et al.*, 1990). From a micro-organism's point of view, meat is an ideal food source, a near perfect nutrient environment (Naumann, 1984).

2.2 Meat quality

The reasons why the consumer buys meat form the basis for the meat industry and these are related to the quality of the meat. Meat has prestige value, its taste appears to be especially attractive and it is generally regarded as having high nutritive value. Meat contributes energy, protein and fat as well as bioavailable zinc and iron, vitamin B1 and B2 and niacin. One of the virtues of meat is its iron content and availability. Much of the dietary iron is poorly absorbed but the iron in meat products is not poorly absorbed. Dietary iron is chelated as Fe:chelates and lost but iron in meat products is not only relatively well absorbed itself but appears to assist the absorption of iron from other foods (Bender, 1975).

The muscle function accounts for colour differentiation between muscles. The crimson or red colour of meat is produced by muscle and blood (myo- and haemo-) globin. This forms part of important criteria in judging meat. Muscle colour varies with species, beef being the darkest, pork the lightest and lamb being intermediate. Muscle colour intensity increases with advances in chronological age of the animal (Walters, 1975, Gault, 1991; Monin *et al.*, 1991). The more than 24 million consumers in the country have widely diverging expectations of the product of which their own conception of "value" is the most important parameter, that is the quality and quantity of the product relative to other foods and consumers' commodities. In this "value package" the consumer demands a regular supply of consistently good quality meat at a reasonable price (Foreshaw,1982; Weyers, 1982).

Meat quality is dependent on the meat production chain from conception to consumption (see figure 2.2). It covers properties which can be perceived visually or organoleptically, and which may be influenced by the immediate pre-slaughter, slaughter and post-slaughter handling of the carcass (Monin, *et al.*, 1991).

These qualities of meat (appearance, palatability, nutritive value and processibility) are influenced and finally established in meat during the different stages of the meat production chain -an integrated system covering the entire ran In general the breed, the sex, the diet/feeding system, the degree of maturity (graded A, B and C) and the interaction of these, influence the size of a carcass through the weight achieved at a given degree of fatness (Cuthbertson, 1975).

ge from "conception to consumption" (Fig.2.2).

Meat quality may best be described by Figure 2.1 below:

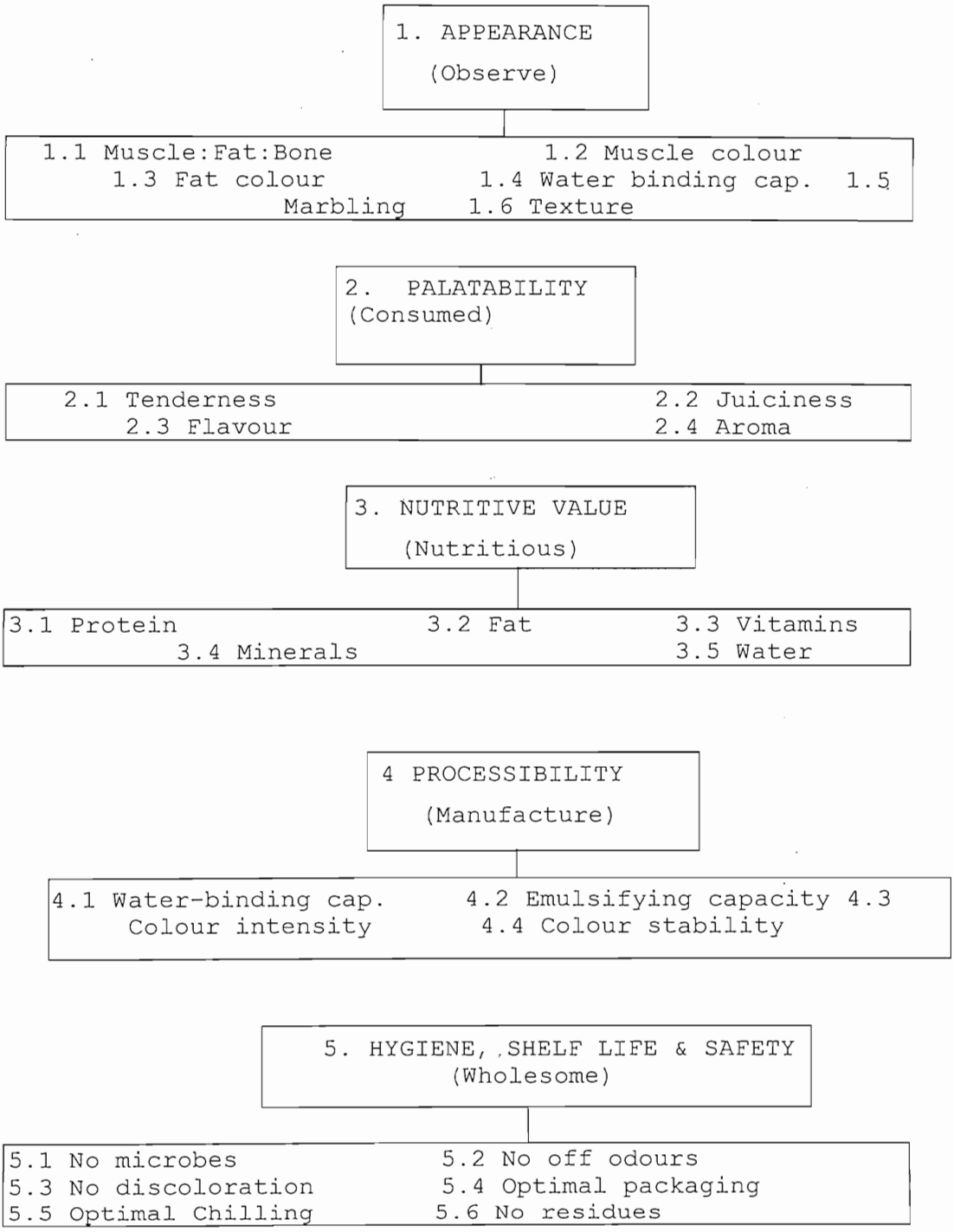


Figure 2.1: Components of meat quality (after Naude, 1984)

Marked yellowness in the fat shows up in contrast to carcasses with whiter fat and it is this contrast with yellow which appears to cause carcasses with yellow fat to be penalised by meat traders. White fat is considered to present evidence of high quality, since yellow fat is usually associated with meat obtained from older dairy cows (Walters, 1975). Yet yellow fat is actually due to prolonged feeding on grasses such as Lucerne (Gracey *et al.*, 1992).

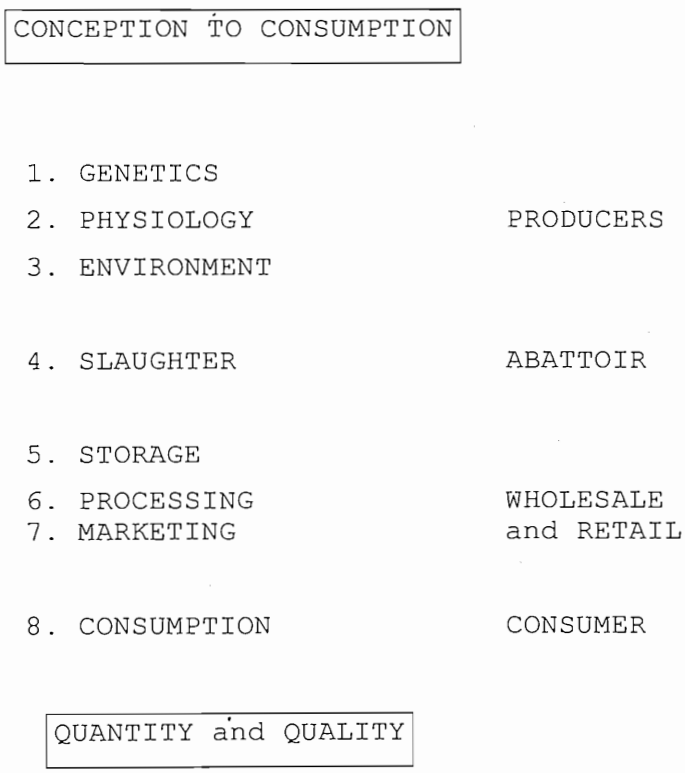


Figure 2.2: The meat production chain (after Nortje, 1992)

Fats give texture, taste, and flavour to foods. Fats also give a satiety feeling.

Water binding Capacity: The moisture content of muscle roughly varies inversely to intramuscular fat (marbling) content. The water holding capacity of beef is said to be negatively correlated with the age (Malterre, 1974; Gaey *et al.*, 1975).

Marbling describes the intramuscular fat. This is found to be in beef, sheep and pigs. Marbling is found to vary inversely to the moisture content of muscle. A high degree of marbling is considered by many to be indicative of good flavour and good eating quality in cooked red meat (Patterson, 1975).

Tenderness: This is very important for the consumer. There has been noticeable differences between breed types. May *et al.*, (1975) found that the heavily muscled Limosin is less tender than Hereford or Simmental crossbreeds. The determination of tenderness involves elements of muscle structure and muscle composition, as well as the nature and the extent of post-mortem biochemical changes. The practice of storing meat after animal slaughter improves its texture in tenderness. On post-mortem storage of meat, toughness increases as muscles go into rigor mortis at about 24 to 36 hrs after slaughter. An exponential decay is then observed and, after ten days storage of the beef at 1°C, meat toughness (as assessed by compressive force) is reduced by about 80% (Patterson, 1975). Electrical stimulation of carcasses has been reported to enhance tenderness and to improve meat colour (Nortje, 1986).

Flavour: Depends on the chemical composition of the meat and on the changes evoked in the latter by cooking and or processing. Water soluble as well as fat-soluble compounds are involved in the development of flavour on cooking (Patterson, 1975). The perception of flavour is a complex sensation, which primarily involves stimulation of the taste receptors in the mouth as well as olfactory receptors in the nasal passages.

Juiciness: This has two organoleptic components in cooked meat. The first is produced by the release of water during the beginning of chewing; the second one is more sustained and due to the stimulatory effect of fat on salivation (Monin, *et al.*, 1991).

Meat and Health: There have been dramatic changes in the carcass composition and preference of the consumer. After World War II in pork manufacture, there has been a shift away from 'fat- type' to ' meat-type' pigs leading to more muscle. Beef carcasses are leaner today than in the past due partly to the infusion of improved genetics for growth and muscling, and because of feeding and management systems to reduce fat accumulation (Reiser *et al.*, 1990). Sheep/Lamb carcasses, however, have become fatter. This is the result of increased confinement, grain- feeding and the lack of proper pricing signals to discourage excess fat production.

The fatty acid composition of meat-fats does not suggest that their consumption will increase blood cholesterol from normal to atherogenic levels. They have actually been shown not to increase blood cholesterol when included in practical diets (Reiser *et al.*, 1990). The intake of excessive amounts of saturated fat, over one's caloric needs however, is an important risk factor in cardiovascular disease, hypertension, stroke, diabetes, obesity, and or specific types of cancer (Briggs *et al.*, 1990). Due to their high oleic acid content meat fats cause an increase in the desirable high density lipoprotein (HDL) (Reiser *et al.*, 1990). Between 1940-1970 meat consumption in the United States of America doubled while colon cancer mortality was unchanged (Kritchevsky, 1990).

The events taking place at and in an abattoir are to a large extent responsible for the wholesomeness of the meat. On arrival and in liarage the animals must be rested. Undue excitement or some psychological or physical stress will lead to poor meat quality due to depletion of glycogen store leading to a high final pH which will influence the processing or keeping qualities of the meat. In pigs, if they are stressed, the pH fall after death is very rapid and this will lead to pale soft and exudative (PSE) pork. The final pH is very important in that it influences colour, WHC as well as keeping quality. In beef a high ultimate pH (in the region of

6 or more) gives rise to dark cutting meat (Dark Firm and Dry (DFD)) which is rejected by consumers (Monin *et al.*, 1991). When there is stress prior to slaughter and the carcass chilled rapidly the result may be PSE pork, DFD beef, pork or lamb and cold shortened tough lamb and young beef (enhanced temperament and behaviour) (Gracey and Collins, 1992).

When the welfare of the animal is not considered as in a private slaughter of an animal, the animal is usually restrained manually and major blood vessels of the neck are severed and the animal bleeds to death. The greater strength of adult cattle makes more forceful restraint essential. This is usually accomplished by use of the restrainer. The animals at the abattoir are immobilised by a stun (electrical at this abattoir) to the head before killing them by exsanguination. The situation in a Moslem or Jewish slaughter is different. In order for meat to be kosher, i.e. right fulfilling the requirements of Jewish law, animals must be slaughtered and dressed according to ritual methods specified in the Talmud, the body of Jewish law and legend based on the Torah which is the substance of God's revelation to man in the Old Testament. (Gracey and Collins, 1991). At slaughter the animal must be alive and healthy and have suffered no injury. Prior stunning of the animal is thus forbidden. Shechita (the act of killing for food) is performed by a Shochet who slaughters the fully conscious animal with a single, deliberate, swift action of a razor-sharp knife, roughly twice the width of the animal's neck and which is devoid of any notch or flaw and has been examined before the slaughter of each animal. It is thus essential for the neck to be fully extended in order to keep the edges of the wound open and thereby prevent any pain. The neck incision is completed without pause. If the knife receives a nick during shechita the meat is condemned. The shochet (cutter) is normally assisted by a sealer (shomer) who is responsible for putting the kosher mark on the brisket and on edible offal.

Sanitation is the act or process of making things sanitary and that this is characterised by keeping things clean and wholesome. The objectives of meat hygiene are firstly to minimise contamination, to ensure wholesomeness and to reduce the public health hazard posed by the possible introduction of pathogenic bacteria such as *Salmonella* spp, *Listeria* spp. *Escherichia coli* and secondly to inhibit the growth of contaminating micro-organisms. Rapid cooling of carcasses prevents the multiplication of most pathogens (except *Listeria monocytogenes* and *Clostridium botulinum* type E) on the carcass surface. Chilling also maintains wholesomeness by limiting the growth of spoilage organisms. Today, the improved health status of slaughter stock, has allowed spoilage organisms to assume more importance than potential food poisoning agents (Bell and Hathaway, 1996).

Sanitation may imply *physically clean* implying that there is no physically visible dirt and *chemically clean* will indicate situations where there are no chemical contaminants and *microbiologically clean* means free from micro-organisms (bacteria mainly) and/or an undesirable number of bacteria. Micro-organisms are responsible for deterioration of product quality as a result of their metabolic activity which make the food offensive to the senses of the consumer. In a way this is an important warning signal lest people ate large amounts of micro-organisms. Food-borne microbial diseases such as Bovine Spongiform Encephalopathy (BSE) which has led South Africa to ban all Beef imports from the United Kingdom (1996), is a case in point. Another recent cause for concern has been the out-break of *Escherichia coli* food poisoning in the United Kingdom (1996).

2.3. Microbiological quality of meat

When one is looking at the microbiological quality of carcasses one is looking at several aspects:

* What micro-organisms are sought, and how to count them?
* How are samples taken, to pick up the micro-organisms for

  counting and or identification?
* What parts of a carcass to examine?
* When to examine?

2.3.1 How to enumerate the micro-organisms

To get a most reliable indication one would want to know the identity and numbers of all the micro-organisms on the carcass- an impracticality. The best is to make separate estimates of a few organisms or groups of particular hygienic significance. When one tries to culture for pathogenic bacteria they usually are so few that they often escape detection because of problems of sampling and recovery. It is more preferred to search for the more numerous indicator organisms. More commonly one seeks indicators of faecal pollution which reveal the possible occurrence of enteric pathogens, notably the Salmonella group, *Escherichia coli* Str. 0157, etc.(Ingram and Roberts, 1976). The term "indicator organisms" can be applied to any taxonomic, physiological or ecological group of organisms whose presence or absence provides indirect evidence concerning a particular feature in the past (usually recent) history of the sample (Harrigan and McCance, 1976).

Indicator organisms have been used in meat and poultry products to assess three factors: microbiological safety, sanitation conditions during processing, and keeping quality of the product (Tompkin, 1983).

Indicators are used to monitor raw materials, processing conditions, and products at various stages of processing and distribution. The economic incentive accompanying longer shelf life has led industry to also use indicators to try and assess the keeping quality.

Aerobic plate count, coliforms, and *Escherichia* coli are the most commonly used indicators of sanitary quality for meat and poultry products. The U.S.A. Department of Agriculture's approach for products at inspected establishments is to incubate plates for two days at 35°C. This will detect those bacteria derived from human contact, the animals being processed, and equipment contaminated with bacteria from either source. Basically the incubation at 35°C indicates the level of sanitation (Tompkin, 1983).

The keeping quality of meat and poultry products can be predicted by monitoring for microbial indicators of spoilage.

The selection of microbial indicators of spoilage varies with the product. In the case of fresh meats and poultry which undergo spoilage by Gram-negative rods such as Pseudomonads, a total count at 25°C for three days is very useful. The temperature of 25°C allows both psychotrophs and mesophiles which may include pathogenic organisms to grow. A lot of work has been done to determine the types of organisms which may be recovered from fresh meat in abattoirs starting from classical work of Empey and Scott (1939). This work described organisms which on re-classification were found to be species of the *Pseudomonas* group. Other workers have also recovered and identified the organisms they found on fresh red meat. These other workers included Norjte (1987) and Dainty *et al.* (1985) who did a lot of work on the probable course of events in meat spoilage by *Pseudomonas* species and *Brochothrix thermosphacta*.

Microbial contaminants that may be found in meat will also include some species of the following *Clostridium, Bacillus, Aeromonas, Corynebacterium, Vibrio, Staphylo-coccus, Alcaligenes, Proteus, Alteromonas, Listeria, Psychrobacter,* the *Moraxella -Acinetobacter* group, *Kingella*, Micrococcaceae, and *Lactobacillus.* The way in which the contaminants may affect the meat has largely been studied in events taking place with *Pseudomonas* and *Brochothrix* as the contaminants (Dainty *et al.*, 1985).

The components of meat tend to fall into one of three groups when considered as potential nutrients for microbial growth. The major components, proteins and fats, must be degraded before they can be utilised by bacteria. Most of these are insoluble and thus not readily available for microbial attack. The second group is composed of low molecular weight, soluble nitrogenous components. These include) creatine and nucleotides, derived during the development of rigor from creatine phosphate and adenosine triphosphate, respectively

i) amino acids, and

ii)peptides, such as carnosine and anserine. The third group of chemicals is derived from muscle glycogen during the onset of rigor (Gill, 1983)

Members of the *Pseudomonas* genus are common inhabitants of soil, fresh water and marine environments, where their activities are important in mineralization of organic matter. The *Pseudomonas* group of organisms are unaffected by the pH in the range that occurs in meat and at chill temperatures grow faster than competing species (Gill, 1983), preferentially utilize glucose and strongly suppress degradation of amino acids until glucose is exhausted, but are capable of using most of the naturally occurring amino acids for growth. Initially *Pseudomonas* grow at the expense of glucose and do not produce any offensive by-products. When the cell density is in excess of $10^8/cm^2$, the supply of glucose becomes

insufficient to meet the bacterial demand and the microbes begin to attack amino acids. The pH of the meat then rises because ammonia is released as a consequence of amino acid degradation. The *Moraxella/Acinetobacter* group utilises amino acids as preferred substrates when growing on meat. The low pH of normal meat however inhibits many in this group. They will flourish at an elevated temperature because the effects of pH are less pronounced (Gill and Newton, 1982).

On DFD meat, pseudomonads predominate as the spoilage organisms. Glucose can be absent from meat in this condition, thus allowing bacteria to utilise amino acids without delay. Spoilage becomes evident when the bacterial numbers are sufficiently high for undesirable by-products to be formed in quantities which can be detected. This occurs when bacterial numbers are somewhat in excess of $10^6/cm^2$ so aerobic spoilage of DFD meat takes place earlier than in normal meat (Newton *et al.*, 1978).

Many bacteria of concern causing food-poisoning exist as a normal part of the flora of domestic animal surfaces, and these inevitably reach some of the meat surfaces during slaughter and butchery, though modern practices attempt to limit this contamination. In South Africa records for food-related disease have been kept since 1989 (Government Gazette, 1989) and both red meat and poultry have been implicated as the foods responsible for most outbreaks. In many bacterial infections, the characteristic pathology of disease is caused by toxins. The toxins may exert their pathogenic effects directly on a target cell or may interact with cells of the immune system resulting in the release of immunological mediators that cause pathophysiological effects.

There are two main types of toxin that have been described: endotoxin (a component of the outer membrane of Gram negative

bacteria) and exotoxin which are elaborated by both Gram-positive and Gram-negative organisms.

Diagramatically this may be shown as below:

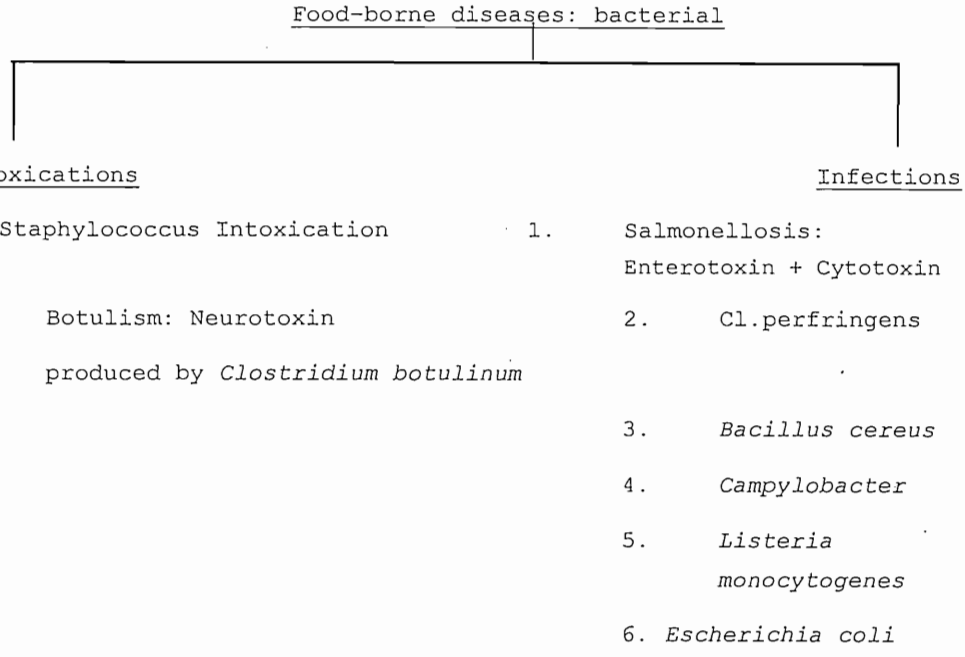


Figure 2.3: The main bacterial food-borne diseases (Frazier, 1988)

Exotoxins are diffusable polypeptides secreted into the external medium by the pathogen. Enterotoxins are exotoxins secreted in the gut and cause symptoms of gastro-intestinal disease, including diarrhoea, dysentery or vomiting. In some cases the disease is caused by ingestion of pre-formed toxin in food but in the majority of cases colonisation of the intestine (requiring a minimum load of micro-organisms for infection to take effect) prior to elaboration of toxin is essential (Cockayne and Arbuthnott, 1992).The cholera toxin and heat labile (L.T.) toxin of enterotoxigenic *Escherichia coli* cause diarrhoea but do not induce inflammatory changes in the intestinal mucosa. They only change the processes regulating ion and water exchange across the intestinal

epithelium (Cockayne and Arbuthnott, 1992). In contrast, the enterotoxins of *Clostridium difficile*, *Clostridium perfringens* type A and *Bacillus cereus* cause structural damage to epithelial cells resulting in inflammation (Cockayne and Arbuthnott, 1992).

Unfortunately the microbes which cause disease in humans are not always associated with obviously spoiled food such as slime, off-flavour, colour change or other visual or olfactory evidence of spoilage even when they are present in high numbers (Brown and Baird-Parker, 1982).

Microbial analysis of samples may also be carried out indirectly by measuring chemical changes. Dainty et al (1985) concluded that a well-defined and reproducible sequence of the production of volatile compounds may be detected in beef stored in air was similar to that frequently recorded for meat stored under gas-permeable films. Compounds produced as a result of microbial growth were acetion, diacetyl, 3-methyl-1-butanol, 2-methyl-1-propanol, ethyl esters of acetic, propionic, butyric, isovaleric and hexanoic acids, methane thiol dimethylsulphide, 1-undecene and 1,4-undecadiene. The first four compounds, which are known end-products of the Gram-positive *Brochothrix thermosphacta* metabolism, were consistently detected at earlier stages of storage than the others which are produced by *Pseudomonas* spp (Dainty *et al.*, 1985).

If one wishes to assess the possible keeping quality of the meat, one estimates the number of micro-organisms capable of growth at refrigeration temperature (psychro-trophs), between 0^{o}-10^{o}C. By using temperatures between 20°-30°C one may estimate mesophiles and psychrophiles and the counts give the most useful general index of over-all hygienic quality (Tompkin, 1983).

The main objectives of meat hygiene are firstly to minimise contamination to ensure wholesomeness and to reduce the public health hazard posed by the possible introduction of pathogenic bacteria such as *Campylobacter* spp.; *Escherichia coli; Salmonella* spp and *Listeria* spp.; and secondly to inhibit the growth of that contaminating micro-organisms (Brown *et al.*, 1982). Most pathogenic contaminants of meat are mesophilic with the exception of *Clostridium perfringens* and *Listeria monocytogenes.* Rapid cooling of carcasses prevents the multiplication of mesophilic pathogens on the carcass surface, thereby reducing the consumer exposure and risk. Chilling also maintains wholesomeness by limiting the growth of spoilage organisms (Bell and Hathaway, 1996).

In a situation where public safety may be at risk it is imperative to have some kind of safe-guard. The safe-guard in a food-producing plant like an abattoir would be the setting down of a product limit and some way of trying to ensure that the limit is adhered to. The International Commission on Microbiological Specifications for Foods (ICMSF) recently held a conference on criteria for microorganisms stating the maximum concentrations of micro-organisms or toxin that can be allowed in a food. The criteria are considered against the possible role micro-organisms may play in foods towards: constituting a health hazard, and possible spoilage. The components of a microbiological criterion should include aspects like sampling techniques, examination methods and microbiological limit(s) (Holzapfel, 1984). A criterion, whether it is a legal limit or administrative regulation and refers specifically to the type(s) and number of organisms not acceptable in a given food, is referred to as micrbiological standard. Because of the variations in distribution of microbes it is unreasonable to operate a standard without a tolerance. The variables scheme uses standards with two levels of concern:

(i) Safety/Quality limits are the upper ones representing the microbial concentration above which a product is unacceptable to both the producer and the consumer; and

(ii) Good Manufacturing Practice (G.M.P.) limit, is the tighter standard and is used within the factory to monitor production. The food producer may want to operate this limit to provide evidence that his processing is under control and that microbiological quality of his batch of food has not deteriorated compared to previous batches. A suitable standard would be that achieved in a hygienic processing plant using good (written down) operating procedures and satisfactory raw materials. The producer can then compare the microbiological quality of his food batches against his GMP standards, and when results indicate that his batch is outside these limits he is able to take corrective action before he exceeds the safety/quality limits (Brown, 1982). A micro-biological specification is the maximum acceptable number of micro-organisms or of specific types of organisms as determined by prescribed methods, in a food being purchased by a firm or agency for its own use or this may be an in-house specification. External specifications resemble standards in that there are penalties for exceeding them and these can take the form of outright rejection of the batch or of discounting of the purchase price.

Hazard Analysis and Critical Control Point (HACCP) refers to a system for monitoring the hygienic performance of production units and especially processing lines so that the hazards associated with particular stages of a process can be recognized, checked and controlled on a routine basis (Harrigan and McCance, 1976; Holzaphel, 1984). The HACCP approach to quality control shifts the emphasis from the final product testing to process and raw material control (Holzapfel, 1984).

2.3.2 How to get the Micro-organisms off for counting

The best method to obtain bacterial representative samples is to cut away the surface (excision) which can then be homogenised with a suitable diluent (usually 0.1% peptone and 0.85% saline) then cultured on a suitable medium and incubated. On a commercial basis excisions have several restrictions: they are restricted to comparatively small areas; laborious, prohibitively damaging to a carcass and not possible with some surfaces. For large-scale survey work one prefers methods which are simpler and do not damage the surface: scraping, swabbing, adhesion or wash-off. A modification of the adhesion - the Agar Sausage technique was found to be the closest an estimate to the excision technique (Nortje *et al.*, 1981).

2.3.3. What parts of the carcass to examine?

The differences from site to site are, however, not consistent from carcass to carcass. Preliminary work carried out at the abattoir as well as that by Nortje *et al.*, (1982), and Meara *et al.*, (1977) found out that beef samples were best sampled from the neck region as well as the fore parts of the carcasses.

2.3.4. When to sample?

The bacteriological condition of the tissues of an animal begins to change greatly and rapidly from the moment of slaughter, hence different pictures are revealed according to the time of examination. The progression of these pictures depends on circumstances especially of temperature and humidity.

The question then is at what point is the best indication of hygienic quality obtained? One suggestion is to examine as soon as slaughter is completed, when the chance influences of external circumstances have had least opportunity to confuse the issue. But, because cooling is the final and essential step before a carcass leaves a slaughterhouse, another suggestion is to sample/ examine at the end of the cooling period.

The latest trend is to monitor process control using the HACCP scheme (Harrigan and McCance, 1976).

2.4. Objectives

In the North West Province there is, as far as I am aware, no documentation available from commercial abattoirs giving information on the range and proportions of micro-organisms as well as the likely consequence of those organisms on fresh red meat.

With this in mind the objectives of the study were to:

a) recover micro-organisms from fresh carcasses and to identify the predominant ones;

b) evaluate the sanitary conditions along a red meat (cattle) slaughter line; and to

c) Correlate information on the identified organisms and the likely effects these may have on the final product (the carcass or the consumer).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Abattoir

The red meat abattoir from which samples were collected is rated as a C50 abattoir. Abattoirs are graded according to the number of beasts they are designed to handle (without some health risk) per week as follows:

Abattoir grade E:- To slaughter 5 Units (usually rural)

D:- To slaughter 15 Units

C:- To slaughter 50 Units

B:- To slaughter 200 Units

A:- To slaughter 1000 Units (Exporting type, such as Kato Ridge and Botswana Meat Commission).

- A unit is the same as 1 bovine, 3 calves, 5 pigs or 15 sheep.

3.1.2. Cattle

The animals are brought in from the various farms or butcheries, usually the day before and are rested in holding pens overnight before the slaughter. At the time of slaughter the animals are driven in a high-walled, smooth-surfaced and narrow passageway into the V-restrainer (Frederiksen's restrainer) box. The stunning box is such that only one animal can be accommodated at any one time and the beast is not visible to the other animals (Butchner, 1974). After stunning

a horizontally hinged flap on the side of the box is released and the animal is ejected. The animal is then shackled and hoisted by one leg, moved to a position above the exsangiunation trough and the main neck blood vessels are severed as quickly as possible (to prevent the return of sensitivity) and the blood is allowed to drain into the bleeding tunnel (Leach, 1992). A typical sequence of operations on a slaughter line after the exsanguination trough would be:

i) removal of the fore then hind legs;

ii) skinning of the hind legs;

iii) skinning of the first half of the stomach chest and the neck and then skin the second half;

iv) skin the right fore-legs;*[1]

v) remove and hang the head;

vi) remove superficial fat over the lower abdomen and eviscerate it;

vii) eviscerate the thorax together with the removal of peritoneal fat;*[2]

viii) split the carcass;

ix) remove the tail and channel fat

x) give the carcass a wash, have it inspected and graded

xi) weigh and tag*[3]and

xii) transfer to the chilling rooms.*[4]

*During these operations samples were taken along the slaughter line at the positions marked by * and the number represents the slaughter line position of the sampling.*

The animal is then skinned by a hand-held electrically driven knife. The brisket is opened by a manually operated, electrically powered saw and the abdominal cavity opened by a slaughterman's knife. The skin of the animal is then removed

from the top down. The carcass is then split into two down the middle by another manually operated, electrically powered chain saw. The carcasses are then washed, graded and inspected. After inspection the carcasses are weighed and moved onto the chillers.

3.1.3. Agar sausages

The modified agar sausage technique was used since it is the least time consuming procedure to perform, no damage is inflicted on the carcass and the results obtained can be expressed as the relative number of organisms present per square centimetre (Nortje *et al.*, 1982). When compared with other sampling procedures such as the double swab technique and other impression or rinse techniques, the agar sausage technique's results are the closest approximation to excision (Nortje *et al.*, 1982). Dorsa et al. (1996) have alluded to the fact that excision gives the best recovery of bacteria. However, excision is a destructive method of sampling and would not be acceptable in a commercial abattoir (Ingram and Roberts, 1976).

3.2. Methods

3.2.1. Agar sausages

The front ends of several five-hundred millilitre (500ml) polypropylene syringes were removed. The syringes were then cleaned and covered with heavy duty aluminium foil and then autoclaved at 121°C 5(five) minutes. The syringes were then placed on a special rack designed specifically for the

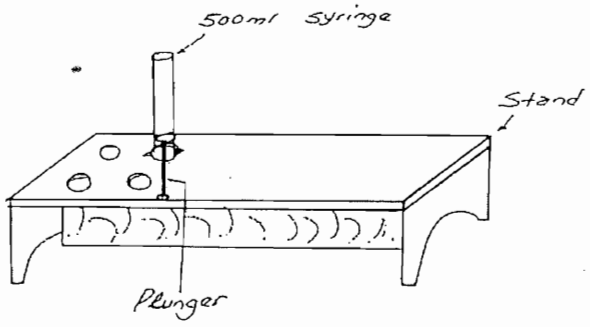


Figure 3.1: A syringe rack and a syringe for the preparation of agar sausages.

syringes such that the plunger of the syringes hangs floating while the base of the bulb of the syringe hangs on top of the rack as shown in Figure 3.1 above.

Thirty one grams (31g) of nutrient agar (Merck) whose composition is in Appendix A, plus an additional 5g agar (to further solidify the product) were weighed up and a litre of distilled water was added to the medium. This was allowed to stand for about fifteen minutes. The suspension was then brought to the boil with constant stirring until it was completely dissolved. This was sterilised by autoclaving at 121°C for 15 minutes. While still in the liquid state, but not too hot, this was poured into the sterile upright facing 500ml syringes and immediately covered by foil and then placed in the refrigerator (at ca. 6°C) for later use.

3.2.2. Sampling

3.2.2.1. Sampling Sites

A pilot survey had been carried out to determine the

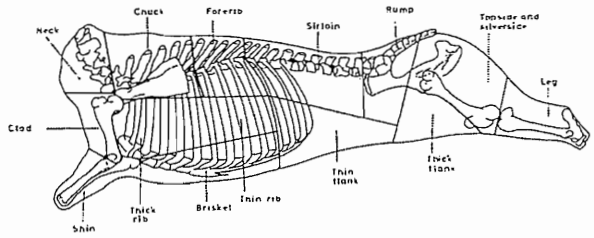


Figure 3.2: A carcass half showing cut and sampling positions

approximate parts of a carcass from which one consistently recovered large numbers of bacteria from the carcasses. The survey indicated that the sampling has to be done from the fore-quarter especially from the neck, the brisket and the fore-rib (Meara *et al.*, 1977; Nortje *et al.*, 1982; Buys *et al.*, 1994). Air samples of the abattoir were not taken since they do not play a significant role in carcass surface contamination (Eustace, 1981).

3.2.2.2. Sampling

Four agar sausages, along with a portable spirit lamp, a sterile knife (covered in foil and previously sterilised for five minutes), sampling bags ((PBI) 500ml Sto-bags DHK.Pty Ltd.), five gas jars on which the agar sausages stood, as well as some 500ml of absolute ethanol were required on each sampling trip.

While working alone, at the sampling point, the knife and the agar sausages were individually flame-sterilised with ethanol. The agar sausage was then brought into direct contact with the carcass surface being sampled, with the application of some mild pressure. A slice of the agar sausage of about 1.5 to 2

mm thickness was cut off and placed into a sterile Sto-bag. This was repeated three times at each sampling site. After each sampling the agar and the knife were flame-sterilised and three samples (each) were taken from the neck area, the breast area and the rear chest area (brisket). The samples were taken as *x*N1, *x*N2, *x*N3 and *x*N4 where *x* represents the carcass sample number; N=neck region and 1 to 4 represent positions on the slaughterline where 1=after skinning, 2=after evisceration, 3=after the final wash and just before weighing and 4=after the chilling for about 18 hours.

3.2.2.3 Sample handling

Each Sto-bag with its contents was weighed to the nearest hundredth of a gram. One hundred milliliters of dilution liquid made up of 0.1% peptone (w/v) and 0.85% (w/v) sodium chloride was added to the sample (Whelehan *et al.*, 1986). The sampling bag and its contents were then homogenised using a stomacher (Colworth 400 from DHK (PTY) LTD) for exactly two minutes (Sharpe, 1972). The samples were then placed in a refrigerator at 7°C. After about 18 hours chilling, sampling was repeated as previously described on the same marked carcasses, placed in sample bags, weighed, homogenised and placed in a cooler box at a temperature of ca. 7°C for transportation to the main laboratory.

At the main laboratory the samples were serially diluted in dilution solution and plated, in duplicate, on pre-dried nutrient agar (Biolab), desoxycholate citrate agar (D.C.A.) (Biolab), Pseudomonas agar base (Oxoid) with the supplement cetrimide-fucidin-cephaloridine (C.F.C.) (Oxoid) and on deMan-Rogosa-Sharpe (M.R.S.) agar (Biolab) media. Each one of the plates was divided into four quadrants so that onto each quadrant a sample of a particular concentration level from the serial dilutions was inoculated. Fifteen microlitres of the sample were placed on each quadrant. The sample was then

spread by the spread plate method using a sterile glass spreader (Nottingham *et al.* 1975).

In order to pick up mesophiles as well as psychrophiles the samples were cultured at 25°C for 48-72hrs (Nottingham *et* al., 1975; Ingram *et al.*, 1976; Nortje *et al.*, 1990). Colony forming units were counted with a "dark-field" Quebec Colony Counter (Model 3328). Representative colonies were streaked on pre-dried nutrient agar plates for isolation and identification. The isolated single colonies (or colony forming units) were then stored on nutrient agar slants for later identification.

3.2.3. Identification of isolates

The isolates on the agar slants were streaked out on pre-dried nutrient agar plates. These were then incubated at 25°C for 24-36hrs. Several stain reactions as well as some phenotypic tests were carried out.

3.2.3.1. Staining reactions

a) Gram stain

The Burke's Gram reaction of the isolates as a first step was carried out for each isolate according to Cruickshank *et al.*, (1980).

b) Endospore staining

Endospore staining was done according to Shaeffer and Fulton (Salle *et al.*, 1973).

c) Flemming's negative staining

Negative staining following the method of Harrigan *et al.* (1966) was done.

d) The acid-fast test according to Ziehl-Neelson's method

The acid fast test was done following the procedure by Cowan (1981).

3.2.3.2. Transmission electron microscopy (T.E.M.)

From the agar slants the isolates were streaked out on nutrient agar. After 18 to 24 hrs the cultures were taken to the laboratory for the electron microscope.

A drop of distilled water was placed on a clean labelled slide. Using a flame-sterilised loop a single colony was picked up and made into a suspension in the distilled water. The palladium side of the grid is the side which is covered by formvar, a plastic coating which acts as a covering of the grid on which the bacteria will adhere. A sample of the organism is then picked up onto the grid using the palladium side of the grid. The grid is then dried using a filter paper to rid it of excess water. The sample is then stained with 0,5% uranyl acetate and immediately dried with filter paper. When working with a large number of samples the grid is placed in the grid-holder, then taken to Phillips CM 10 transmission electron microscope for viewing.

3.2.4. Phenotypic tests

a) Oxidase test

A piece of filter paper was wet with a freshly prepared one percent solution of tetramethyl-p-phenylene diamine-dihydrochloride. The test culture was then smeared over the paper by means of a platinum needle. If it turned purple within 5-10 seconds it was referred to as a positive reaction, if the purple colour developed after 10 seconds but not after 60 seconds it was called a delayed positive. After 60 seconds the reaction was regarded as negative (Cruickshank *et al.*, 1980; Brock *et al.*, 1991).

b) Catalase test

The majority of organisms growing in the presence of free oxygen will show a positive catalase reaction. The test was done using 3% hydrogen peroxide(Harrigan and McCance, 1976; Salle, 1973; Cruickshank *et al.*, 1975; Brock *et al.*, 1991).

c) Respiratory-fermentation tests (RF)

(This used to be called the Hugh and Leifson Oxidative and Fermentative test, 1961). Two tubes per sample containing the semi-solid basal medium with one percent carbohydrate, in our case, and usually, glucose, and bromocresol purple indicator were inoculated by stab culturing. One tube was immediately covered with sterile liquid paraffin to ensure anaerobic conditions. Strict aerobes such as *Pseudomonas* spp would only form acid in the uncovered tube. Facultative anaerobes such as enterobacteriaceae will form acid in both the covered and the uncovered tube through the processes of respiration and fermentation (resp.). The results were observed daily for a

period of seven days (Cowan *et al.*, 1981, Cruickshank *et al.*,1980; Brock *et al.*, 1991).

d) Anaerobic growth test

From 24-hr pure culture samples were streaked on pre-dried nutrient agar and placed in anaerobic jars with GasPak Catalyst Replacement charges (for an anaerobic environment). The test control organisms were an obligate aerobic bacterium (*Pseudomonas aeruginosa*) and a facultative anaerobic bacterium (*Escherichia coli*) and were placed together with the test samples. The anaerobic jars were connected to a vacuum pump for about ten minutes each to evacuate all the air. They were then filled with the anaerobic air which consisted of 20,3% hydrogen gas; 9,8% carbon dioxide and 69,9% nitrogen gas. The flasks were then sealed and incubated at 20°C. After a week the flasks were opened and observed. The results from the anaerobic growth test were then compared with those observed from the RF test.

3.3. Motility test

Using the motility medium (see Appendix A) the isolates were tested for motility and the results observed were compared with those obtained from the results of the T.E.M. (whether those isolates which appeared motile did have any flagella at the T.E.M.). In cases where motility was observed but no flagella observed young cultures were prepared and observed under the T.E.M. after an incubation period of 18 hours.

The results from all the tests were used in drawing up tables from which deductions, using the keys of Dainty *et al.*,(1985) as well as the Fischer identification keys (1986) were made about the isolated organisms. Confirmation where the identity

of an organism was not clear was obtained from Balows *et al.*, (eds. 1992).

3.4. Statistical analysis

The collected raw data were converted to $\log_{10}$ and tested analyses of variances were calculated using the *Statistica* and *SAS* to determine whether there was statistical significance in the findings.

CHAPTER 4

QUANTIFICATION AND IDENTIFICATION OF ISOLATES

4.1 Introduction

The main purpose of this project was to survey the relative bacterial numbers along a red meat (cattle) slaughter line. The slaughter line positions were designated 1, 2, 3 and 4 and these correspond to 1: after skin removal (flaying); 2 after removal of internal organs (evisceration); 3 after the final wash and 4 after about eighteen hours of chilling. Samples were collected using the methods as described in chapter 3.

4.2 Bacterial counts on nutrient agar

4.2.1 Results obtained on Nutrient agar

The raw data of eight samplings were converted to $\log_{10}$ so as to make the values comparable with literature values. The individual results from each sampling visit were treated as replicates and averaged out for each position. The results are given in Table 4.1.

Table 4.1: The log of the average bacterial counts on nutrient agar.

	NECK		FORE-RIB		BRISKET	
POSITION	Means	Std.Dev	Means	Std.Dev	Means	Std.Dev
G_1:1	3.09	1.02	2.72	1.23	2.89	1.20
G_2:2	3.38	0.77	2.99	1.07	2.98	0.86
G_3:3	2.96	1.15	3.13	1.21	2.80	1.26
G_4:4	2.73	0.97	2.33	1.06	2.54	0.71
All Grps on N.A.1	3.06	0.97	2.80	1.13	2.81	1.01

In the Table above: G_1:1 represents the average of all the plate counts at position 1 (after skin removal)

G_2:2 represents the average of all the plate counts at position 2 (after evisceration)
G_3:3 represents the average of all the plate counts at position 3 (after the final wash) and
G_4:4 represents the average of all the plate counts at position 4 (after ca. 18 hours chilling).

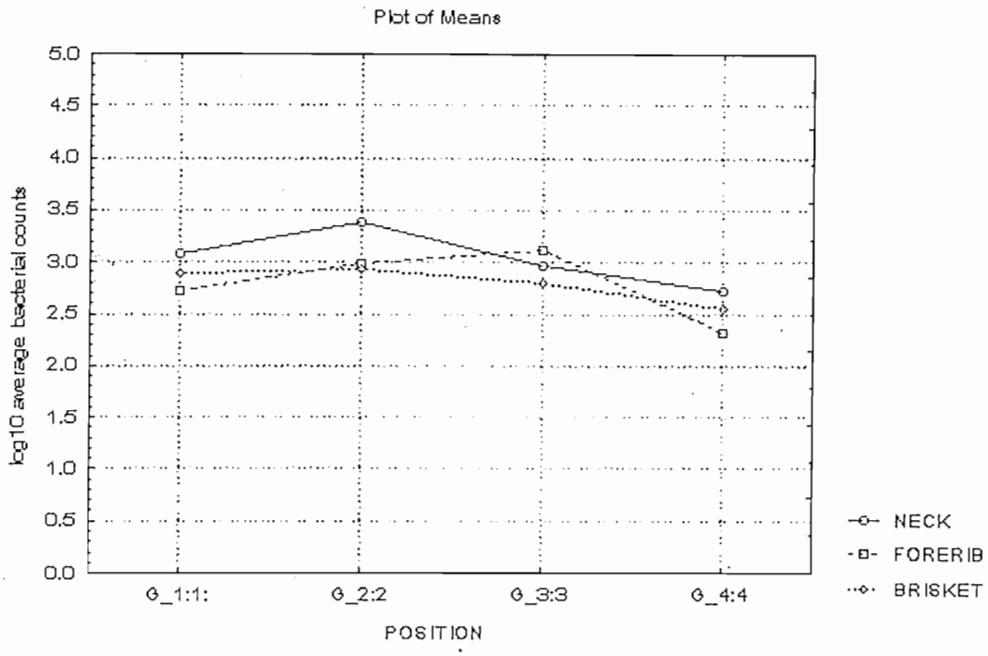


Figure 4.1: A graph of the average bacterial counts on nutrient agar vs the relative positions along the slaughter line.

For the neck and the brisket when superficially viewed there appears to be a slight decrease in numbers as one goes along the line. There did not seem to be any real change for the fore-rib. Position 2 seems to have high counts. From the calculated means and the standard deviations, the coefficient of variation (c.v.) of the data was worked out and the following table (Table 4.2) shows the results obtained.

Table 4.2: One way analysis of variance of the bacterial counts observed on N.A. per position.

Variable	SS Effect	d.f. Effect	MS Effect	SS Error	d.f. Error	MS Error
NECK	1.98	3	0.66	30.79	32	0.96
FORE-RIB	2.66	3	0.89	34.14	26	1.31
BRISKET	0.69	3	0.23	33.95	31	1.09

Variable	F	p
NECK	0.69	0.57
FORE-RIB	0.68	0.58
BRISKET	0.21	0.89

effects are significant at $p < 0.0500$

When results are statistically significant this is a convention for describing the situation when the 'p' value is less than 0.05 (Watts, 1993). In my case all the p values were much higher than 0.05 thus there was no statistical significance in the trend from position 1 to 4 or from the sites of the neck, fore-rib or brisket.

When the bacterial counts are the dependent variable and the the position on the slaughter line and the site on the carcass are the idependent variables I was able to do a two-way ANOVA:SAS system. I had to use unbalanced data (to take care of those counts which had zeero counts). This was shown as type III ANOVA.

Table 4.3: Two-way ANOVA:SAS system-unbalanced data of bacterial growth on nutrient agar

Source	DF	Type IIISS	Mean Square	F Value	P value
Position	3	3.5147	1.1715	0.98	0.41
Site	2	0.7691	0.3845	0.32	0.73
Pos*Site	6	1.7007	0.2834	0.24	0.96

Here too the *P* value is only significant when it falls below 0.0500. There was thus no significant relationship between the counts one got and the position along the slaughter line and or the site from which one was sampling.

Table 4.4: Coefficient of variations from the All grps data of growth on nutrient agar 1 above.

	Mean	Std. Dev.	C.V. %
Neck	3.06	0.97	32
Fore-rib	2.79	1.13	41
Brisket	2.81	1.01	36

From the tables it was calculated that the coefficient of variation in all the sampling positions were very high. The high coefficient of variation is an indication that there was wide variation in the individual readings obtained.

From Figure 4.1, the relative counts of the organisms on nutrient agar from the neck region for positions 1, 2 and 4 appeared higher than the counts from the fore-rib and the brisket. This finding conforms with the growths of

the bacteria on desoxycholate citrate agar (see Section 4.3). There appeared to be a considerable increase in the counts from the fore-rib after position III. There was then observed some decrease in the number of organisms recovered after eighteen hours of chilling particularly at the fore-rib position only.

4.3 Bacterial counts on desoxycholate citrate agar

As was the case with the counts on nutrient agar these were also converted to $\log_{10}$. They were then drawn up into a table as shown below

Table 4.5: The log of the average bacterial counts on desoxycholate citrate agar.

	NECK		FORE-RIB		BRISKET	
POSITION	Means	Std.Dev	Means	Std.Dev	Means	Std.Dev
G_1:1	2.14	0.65	1.98	1.13	1.95	1.25
G_2:2	3.05	0.87	2.09	0.99	2.29	1.04
G_3:3	2.21	1.02	2.82	1.09	2.33	1.23
G_4:4	2.12	1.11	1.58	0.91	1.77	1.37
All Grps on DCA	2.38	0.96	2.11	1.06	2.16	1.12

As was the case with the growths on nutrient agar there appears to be a sharp increase in numbers of bacteria recovered from the neck region after evisceration, then there was decrease from position 3 to position 4. The bacterial counts from the fore-rib tend to increase

considerably from position 2 to position 3 then decrease sharply after the chilling process.

Graphically the data of above is represented as shown below:

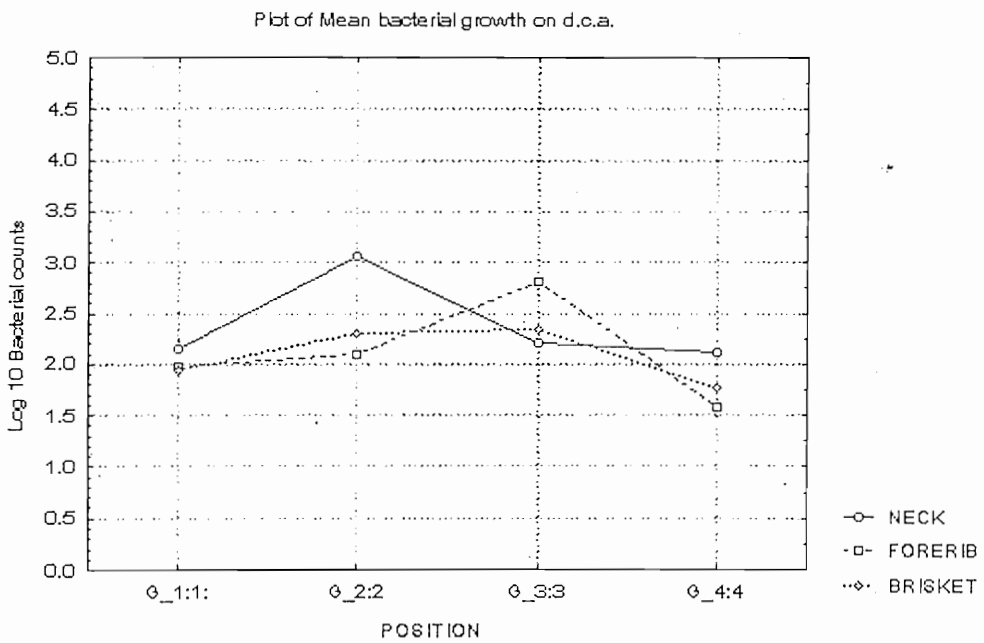


Figure 4.8: A graph showing average $\log_{10}$ bacterial counts on desoxycholate citrate agar at the four positions.

The growth of the bacteria from the brisket region did not show significant increases or decreases. These apparent trends were then tested for by way of statistical evaluation.

The data from the desoxycholate agar cultures were then evaluated to determine statistical significance of the apparent trends.

Table 4.6: Analysis of variance of readings on the desoxycholate citrate agar

Variable	SS Effects	d.f. Effects	MS Effects	SS Error	d.f. Error	MS Error
NECK	4.29	3	1.43	21.7	25	0.87
FORE-RIB	4.04	3	1.35	17.1	16	1.07
BRISKET	1.03	3	0.34	25.5	18	1.42

Variable	F	p
NECK	1.65	0.20
FORE-RIB	1.26	0.32
BRISKET	0.24	0.87

effects are significant at $p<0.050$

The lower values of the *p* values for the neck and the fore-rib, as compared to those of the nutrient agar data suggest some amount of superficial significance though the statistical evaluation does not justify this. This is also observed when one works out a two-way analysis of variance, see table 4.8 below.

Table 4.7: Two-way analysis of variance of bacterial growth counts on DCA.

Source	DF	Type IIISS	Mean Square	F Value	P value
Position	3	3.4238	1.1413	1.60	0.2
Site	2	2.1523	1.07614	1.51	0.23
Pos*Site	6	7.2647	1.2108	1.70	0.13

The two way analysis of the data for the bacterial growth on DCA shows *p* values which are much less than those observed on the nutrient agar this implies more correlation between position and site to the counts observed on the DCA than thatobserved on the DCA, however it was not enough to be significant.

The coefficient of variation from the All Grps data of growth on DCA was worked out as below:

Table 4.8: Coefficient of variation from the All Grps data for growths on desoxycholate citrate agar

Variable	Mean	Std. Dev	C.V.%
NECK	2.38	0.96	40
FORE-RIB	2.11	1.06	50
BRISKET	2.16	1.12	52

The coefficient of variation of the bacterial growths on desoxycholate citrate agar are much wider than on the nutrient agar. This is to be expected and may be related to

the fact that desoxycholate citrate agar is a selective medium and as such will only select for those organisms' growth if they are present they will then grow if those particular organisms are not present then there will not be any growth.

4.4 Isolation and identification of organisms on desoxycholate citrate agar

The isolates from the different parts of the carcass were put to several tests so as to differentiate them. The keys of Fischer *et al.*, (1985) and Dainty *et al.*, (1986) were used as guides.

Table 4.9: Characterisation of the isolates recovered from the desoxycholate citrate agar medium.

Sample Number	Gram Stain	T.E.M. Morphology	Catalase rxn	Oxidase rxn	R.F. Test	Motility	Presumptive
2N2 dca	-	Bacilli	+	-	R.f.	+	Enterobacteriaceae
2N4 dca	non-reculturable						
4N3 dca	-	Bacilli	+	-	Rf	-	Enterobacteriaceae
6L4 dca	-	Bacilli	+	+	N.r.	+	*Pseudomonas*
7L4 dca	non-reculturable						
9N1 dca	-	Bacilli	+	+	N.r.	+	*Pseudomonas*
9L1i dca	non-reculturable						
Sample Number	**Gram Stain**	**T.E.M. Morphology**	**Catalase rxn**	**Oxidase rxn**	**R.F. Test**	**Motility**	**Presumptive**
9L1ii dca	-	Bacilli	+	+	N.r.	+	*Pseudomonas*
9B1i dca	-(fat)	Bacilli	+	+	N.r.	+	*Pseudomonas*
9B1ii dca	-	Bacilli	+	-	R.f.	+	Enterobacteriaceae

9L1 iii dca	-	Bacilli	+	++	N.r.	+	*Pseudomonas*
9N2 dca	-	Cocco-bacilli	+	+	N.r.	-	*Moraxella*
9L2 dca	-	Bacilli	+	+	R	+	*Pseudomonas*
9N3 dca	non-reculturable						
9B2 dca	-	Bacilli	+	+	N.r.	+	*Aeromonas*
9B3 dca	-	Bacilli	+	+	N.r.	+	*Aeromonas*
9L3 (i) dca	-	Bacilli	+	+	R.	-	*Pseudomonas*
9L4 dca	-debris++	Bacilli	+	+	R	+	*Pseudomonas*

Table 4.10: Ratios of microbial isolates from positions along the slaughter line cultured on desoxycholate citrate agar.

	Position 1 Isolates		Position 2 Isolates		Position 3 Isolates		Position 4 Isolates		TOTAL Isolates	
Genera	Num.	%	Num.	%	Num.	%	Num.	%	Num.	%
Pseudomonas spp.	4	67	3	50	1	25	2	50	10	50
Moraxella spp.	0	0	1	17	0	0	0	0	1	5
Enterobacteriaceae	1	17	1	17	1	25	0	0	3	15
Aeromonas spp	0	0	1	17	1	25	0	0	2	10
Non-reculturable	1	17	0	0	1	25	2	50	4	20
TOTAL	6		6		4		4		20	

Of the twenty isolates half of them were *Pseudomonas* spp and 20% were non reculturable. The information in the table above can best be represented by a pie chart as shown below:

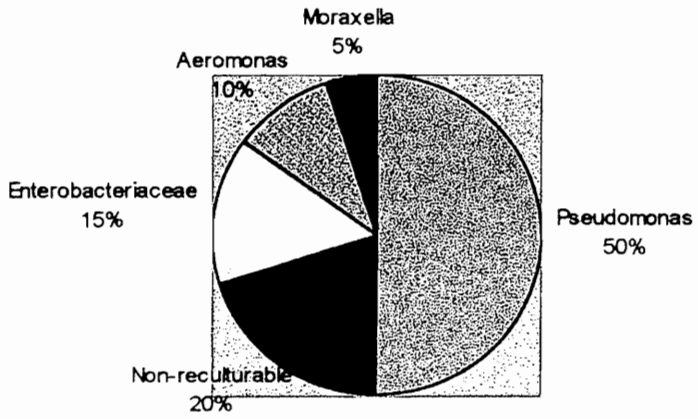


Figure 4.3: Microbial groups identified as percentages grown on desoxycholate citrate agar.

The general log counts of about three log numbers on nutrient agar, per square centimeter imply fairly clean and hygienic operations though not as clean as is the case in some studies which were carried out at a research-linked abattoir not a commercial one (Brown *et al*, 1982; Nortje, 1990). The neck region being the lowest of the carcass which is moving on rails was at about the knee level and thus was most susceptible to contamination from the aprons of the workers, as well as from the skin and or hide indirectly via the hands or directly to it. The abattoir operations are labour intensive and from slaughter to chilling the process of on-the-rail dressing is divided into many stages. The skinning of the legs, removal of the hooves, skinning of the stomach, one half at a time, removal and hanging of the head, sawing of the brisket, removal of superficial fat around the adder area and open the perineum and eviscerating the abdomen then the thorax, the carcass is then split by use of a chain saw which is supplied with a fine jet of water to cool as well as to lubricate the cutting process, each of which is undertaken by a separate operator or one operator may do several of these functions and is thus prone to transfer contaminants

from one site to the next. All the peritoneal fat is placed in one bucket, while the offals are placed in another bucket. Aborted foetuses are removed and discarded. The average counts from the fore-rib conform to this by showing an increase in the counts up to sampling site 3.

No growths were observed on the M.R.S. agar. The fact that the samples were fresh and that there was no anaerobic atmosphere at the site and at the time of sampling was probably the reason why I did not get any growths.

4.5 Isolation and Identification of organisms on nutrient agar

The isolates from the different parts of the carcass at specific designated positions along the slaughter line were subjected to a few tests in order to differentiate them. The keys of Fischer *et al.*, (1985) and Dainty *et al.* (1986) were used for the identification of the isolates. The results are given in Table 4.11. and confirmation was sought from Balows *et al.* (1992).

Table 4.11: Characterisation of the all the isolates collected.

Sample Number	Gram Stain	T.E.M. Morphology	Catalase Rxn	Oxidase Rxn	R.F. Test	Motility Test	Presumptive
2B1	-	Bacilli	+	+	R.f.	+	*Aeromonas*
2B2	+	Strepto-bacilli	+	+	R	-	*Brochothrix*
2B3	+	Cocci	+	+	N.r.	-	Micrococcaceae

Sample Number	Gram Stain	T.E.M. Morphology	Catalase Rxn	Oxidase Rxn	R.F. Test	Motility Test	Presumptive
2T1	-	Bacilli	+	-	N.r.	-	*Acinetobacter*
2T2(i)	-	Cocci	+	+	N.r.	-	*Moraxella*
2T2(ii)	+	Cocco-bacilli	+	+	N.r.	-	Micrococcaceae
2N4(i)	+	Cocci	+	-	R.f.	-	*Staphylococcus*
2N4(ii)	-	Bacilli	-	+	Rf.gas		*Kingella*
3N1							
3N2	-	Cocco-Bacilli	+	-	N.r.	-	*Acinetobacter*
3N3	Non-reculturable						
3B1	-	Cocco-Bacilli	+	-	N.r.	-	*Acinetobacter*
3n4(i)	-	Bacilli	+	+	Rfgas	+	*Aeromonas*
3N4(ii)	- (fat)	Bacilli	+	-	R.f.	-	Enterobacteriaceae
4N1	-	Bacilli	+	-	R.f.	-	Enterobacteriaceae
4L2 (i)	+	Cocci	+	-	R.f.	-	*Staphylococcus*
4L2(ii)	+	Cocci	+	-	R	-	*Staphylococcus*
4N3	-	Bacilli	+	+	N.r.	+	*Pseudomonas*
4N3(iii)	-	Bacilli	++	+	R.f.	+	*Aeromonas/Vibrio*
4N3 i) b	-	Bacilli	-	+	N.r.		*Kingella*
4B3 I) y	- (fat)	Bacilli	+	+	R	+	*Pseudomonas*
4N3 ii)	- (fat)	Bacilli	+++	+	Rgas	+	*Pseudomonas*
4B3 ii)	- (fat Yl)	Bacilli	+	-	R	-	*Pseudomonas*
5L3 I	-	Bacilli,	+	-	R.f.	-	Enterobacteriaceae
5L3 ii	Non-reculturable						
5L3	Non-reculturable						
6N1	-	Bacilli	+	++	R.f.	+	*Aeromonas / Vibrio*
6B2	-	Cocco-Bacilli	+	-	N.r.	-	*Acinetobacter*
6B3	Non-reculturable						
6N3	Non-reculturable						

Sample Number	Gram Stain	T.E.M. Morphology	Catalase rxn	Oxidase rxn	R.f. Test	Motility test	Presumptive
6L4	-	Bacilli	+	+	N.r.	+	*Pseudomonas*
7N2	-	Bacilli	+	+	N.r.	-	*Pseudomonas*
7N4	-	Bacilli	+	+	N.r.	+	*Pseudomonas*
7N4 ii)	Non-reculturable						
7B3 .	+ (big)	Cocci	+	-	N.r.	-	Micrococcaceae
7L1	+	Cocci	+	-	N.r.	-	Micrococcaceae
7L4	-	Bacilli	++		N.r.	+	*Pseudomonas*
7L1(2)	- (thin)	Bacilli	+	+	N.r.	+	*Pseudomonas*
7B3 (ii)	-	Bacilli	+	+	R	+	*Pseudomonas*
7L2	-	Bacilli	+	+	R	-	*Pseudomonas*
8B1a)	-	Bacilli	+++	+	R.f.	+	*Aeromonas / Vibrio*
8L1	+(big)	Bacilli	++	+	N.r	+	*Bacillus*
8B1d)	-	Bacilli	+	+	R.f.	+	*Aeromonas / Vibrio*
8N1a)	-	Bacilli	+	+	R.f.	+	*Aeromonas / Vibrio*
8B1c)	-	Bacilli	+	+	R.f.	+	*Aeromonas / Vibrio*
8B2 i)	-	Bacilli	+	+	R.f.	+	*Aeromonas / Vibrio*
8N2ii)	-fat	Cocco-Bacilli	+	-	N.r	-	*Acinetobacter*
8N3	+ short	Bacilli	+	+	N.r	-	*Bacillus*
8N1b)	-slime++	Bacilli short	+	+	R.f.	+	*Aeromonas*
9N2 i)	-	Bacilli	+	+	N.r	+	*Pseudomonas*
9L1	-(tiny)	Bacilli	+	+	N.r	-	*Pseudomonas*
9N1c	-slime++	Cocci bacilli	+	+	N.r	-	*Moraxella*
9N1 y	-slime++	Bacilli	+	+	N.r		*Pseudomonas*
9L1 (3)	-	Bacilli	+		N.r		*Pseudomonas/ Alteromonas*
9N2 ii)	-(fat)	Cocco-Bacilli	+	-	N.r	-	*Acinetobacter*
9N1	-	Strepto-bacilli	+	+	N.r	-	*Alteromonas / Pseudomonas*
9B3 (i)	-(V.fat)	Bacilli	+	+	N.r	-	*Psychrobacter*

Sample Number	Gram Stain	T.E.M. Morphology	Catalase rxn	Oxidase rxn	R.f. Test	Motility test	Presumptive
9L3 (i)	- (small)	Bacilli	+	+	N.r	+	*Alteromonas*
9B3(ii)	- (oval)	Bacilli	+	-	N.r	+	*Acinetobacter*
9L3(iii)	-(smelly)	Bacilli	+	+	N.r	+	*Alteromonas*/ Pseudomonad
9B4 (i)	- v.Smelly	Cocco-bacilli	+	+	N.r	-	*Moraxella*
9L4	Non-reculturable						
9B4 (2)	-	Cocco-bacilli	+	+	N.r	-	*Moraxella*
10B1(3)	+orange	Bacilli	+	-	N.r	-	*Corynebacterium*
10B1(1)	- long/fat	Bacilli	+	-	N.r	-	*Pseudomonas*
10N2(2)	-stinking	Bacilli	+	+	Rfgas	-	*Aeromonas*
10N2(1)	-	Bacilli	+	-	N.r	-	*Pseudomonas*
10N2(3)	Non-reculturable						
10N2(2) Y	-	Bacilli	+	+	R	-	*Pseudomonas*
10B2	-	Bacilli	+	-	N.r	-	*Pseudomonas*
10B3	+Sarcin a	Cocci(Huge)	+	-	N.r	-	Micrococcaceae
10N3(1)	-slime++	Bacilli	+	+	N.r	-	*Pseudomonas*
10N3(2)	-	Cocco-bacilli	+	+	N.r	-	*Moraxella*
10N3(3)	- motile?	Bacilli	+	+	N.r	-	*Pseudomonas*
10N3(4)	-	Bacilli	+	+	R.f.	+	*Aeromonas*
10L3(1)	- small	Bacilli	+	+	Rgas	-	*Aeromonas*
10L3(2)	- slm balls	Bacilli	+	-	N.r	+	*Pseudomonas*
10N4(1)	+ with inclusion s	Cocci	-		N.r	-	Micrococcaceae
10N4(2)	+	Cocci	+	-	N.r	-	Micrococcaceae
10N4(4)	-(Long)	Bacilli	+		N.r	-	*Pseudomonas*
10B4 yellow	-	Tetrads /Diplococci	++	+	N.r	-	*Moraxella*
10L4	+slime++	Bacilli	+	+	N.r	-	*Corynebacterium*

Sample Number	Gram Stain	T.E.M. Morphology	Catalase Rxn	Oxidase Rxn	R.F. Test	Motility Test	Presumptive
10L4(3)	Non-reculturable						
11L1(1)	+palisade	Bacilli	+	-	N.r	-	*Brochothrix*
11L1(2)	-V.long	Bacilli	+	-	R	-	*Pseudomonas*
11L1(3)	+	Cocci	+	+	N.r	+	Micrococcaceae
11L1(4)	+slime++	Cocco-bacilli	+	-	Rf	-	*Brochothrix*
11N1	- small	Bacilli	+	+	N.r	-	*Pseudomonas*
11B1(1)	+sml.oval	Bacilli	+	-	R.f.	-	*Brochothrix*
11N2(1)	+small	Bacilli	+	-	N.r	-	*Corynebacterium*
11N2(2)	-	Bacilli	+	+	N.r	+	*Alteromonas*
11B2(1)	- Staphylo	Cocci-bacilli	+	-	N.r	-	*Acinetobacter*
11B3(1)	- Y. hard	Bacilli	+	-	N.r	-	*Acinetobacter*
11B3 (2)	- capsule ?	Bacilli	+	+	N.r	-	*Pseudomonas*
11L3 (1)	- palisade	Bacilli	+	+	N.r	-	*Pseudomonas*
11L3 (2)	-Debri++	Cocci-bacilli	+	+	N.r	-	*Moraxella*
11N3 (2)	- Bubbly Inclusions	Bacilli	+	+	R.f.	-	*Aeromonas/ Vibrio*
11N4 (1)	-	Bacilli	+	+	R.f.	-	*Aeromonas/ Vibrio*
11N4 (2)	+	Strepto-bacilli	+	-	N.r	-	*Brochothrix*
11L4 (1)	+ 4s & 8s	Cocci	+	+	N.r.	-	Micrococcaceae
11L4 (2)	-	Bacilli	+	+	N.r	-	*Moraxella*
11B4 (1)	+	Cocci	+	+	N.r	-	Micrococcaceae

In the table above
T.e.m: is for Transmission electron microscopy;
R.f test: is the respiratory or fermentative test;
N.r: is No reaction observed or inactive;
R.f: implies that the organism can metabolise glucose both fermantatively as well as respiratorily, these are facultative

anaerobes and were seen by production of acid in both the sealed and the open tubes;

F: indicates an organism which can only metabolise glucose anaerobically, these would be strict anaerobes;

R: are organisms which show respiratory activity only, these are strict aerobes.

Y. hard: is to show that the colonies were very brittle and could not be made into a smear easily, tending to move *en masse*.

sml. oval: this was to give the description bacterial cells as small and oval.

In the results table the sample numbers are given as xN2(ii); where the;

x represents the carcass number or the replicate number from 2 to 11 (Sample 1 was discarded because it had been kept for too long (more than 72 hours) in the dilution fluid not used);

N is the part of the body, the neck, which could have been B, the brisket and L is the fore-rib, as shown in the table;

2 is the sampling position along the slaughter line, where it could have been 1 which is just after skin removal, 2 is after evisceration, 3 is after final wash and inspection and 4 is after eighteen hours chilling;

(i) is the first or (ii) the second or subsequent isolates from a particular set of plates.

The information from the table above was used to make out another table showing abattoir position and microbial groups recovered.

Table 4.12: Ratios of microbial isolates from four different sampling positions along the slaughter line.

	Position 1 Isolates		Position 2 Isolates		Position 3 Isolates		Position 4 Isolates		TOTAL Isolates	
	Number	%	Number	%	Number	%	Number	%	Number	%
Pseudomonas spp	7	25	6	30	10	30	4	17.4	27	26.0
Aeromonas spp	7	25	1	5	4	12	2	8.7	14	13.5
Micrococcaceae	2	7.1	1	5	3	9	5	22.0	11	10.6
Acinetobacter spp.	2	7.1	5	25	2	6	1	4.3	10	9.6
Moraxella spp.	1	3.6	1	5	2	6	4	17.4	8	7.7
Bacillus spp.	1	3.6	0	0	1	3	0	0	2	1.9
Alteromonas spp.	1	3.6	1	5	3	9	0	0	5	4.8
Enterobacteriaceae	1	3.6	0	0	1	3	1	4.3	3	2.8
Psychrobacter spp.	0	0	0	0	1	3	0	0	1	1.0
Kingella spp.	0	0	0	0	1	3	1	4.3	2	1.9
Staphylococcus spp.	0	0	2	10.0	0	0	1	4.3	3	2.8
Brochothrix spp.	2	7.1	1	5.0	0	0	1	4.3	4	3.8
Corynebacteria spp.	2	7.1	1	5.0	0	0	1	4.3	4	3.8
Non re-culturable	2	7.1	1	5.0	5	15	2	8.7	10	9.6
TOTAL	28		20		33		23		104	

From this table it was then possible to find out which group of organisms predominated in numbers and how they related to the others at each position.

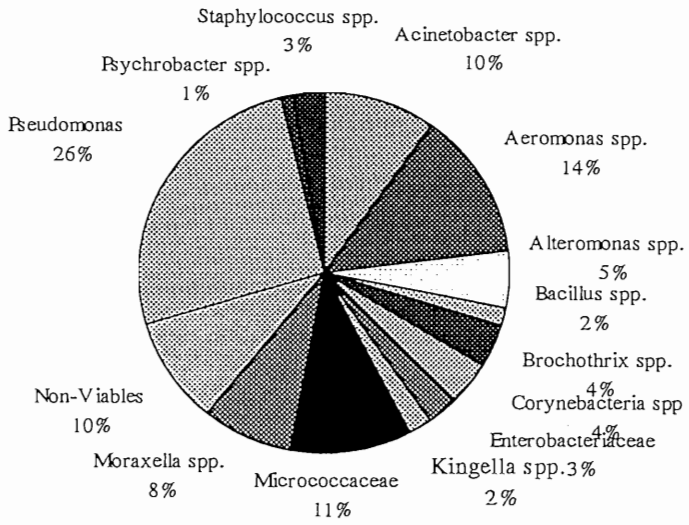


Figure 4.4: Microbial groups identified as a percentage of the total number of the isolates on nutrient agar

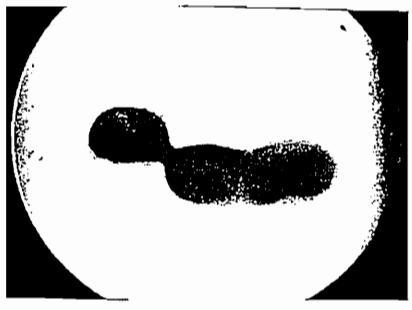
Of the one hundred and four organisms isolated 26% were *Pseudomonas* spp, 13.5% were *Aeromonas* spp and the micrococcaceae made up 11% while the *Acinetobacter* spp made up 9.6%. The *Moraxella* spp, *Bacillus* spp, *Alteromonas*, Enterobacteriaceae, *Psychrobacter* spp, *Kingella* spp, *Staphylococcus*, *Brochothrix* spp and the *Corynebacterium* spp all formed less than nine percent of the isolates each. The ratio of those organisms which could not be re-cultured was nearly ten percent of the total.

Examples of some of the bacteria important to the meat industry are given below:

Sample 7B3(ii)

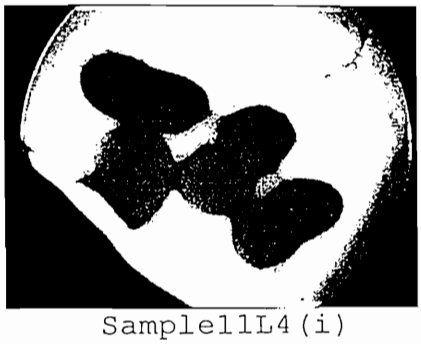
Sample 10N3(iii)

Sample 8N2(ii)



Sample 10B2

Sample 9N2(ii)



Sample11L4(i)

Sample 8N1(i)

Sample 9L3(ii)

Sample 11L1(i)

Figure 4.5: Electron micrographs of examples of some of the isolates.

Samples 7B3(ii) and 10N3(iii) are examples of the *Pseudomonas* spp., 8N2(ii), 10B2 and 9N2(ii) are examples of the *Moraxella/ Acinetobacter* group, 8N1(i) and 9L3(ii) are examples of the *Alteromonas* spp., 11L4(i) is an example of Micrococcaceae and 11L1(i) is an example of a *Brochothrix* spp.

Chapter 5

Discussion and Conclusion

5.1 Introduction

The purpose of the project was to survey the relative bacterial numbers along a red meat slaughter line and to characterize the organisms picked up.

The total aerobic counts offer a sensitive indication of the dressing hygiene standards. In healthy slaughter animals the tissues that will become meat, are generally regarded as being sterile and that any organisms found on its surface are a result of contamination from a variety of sources, such as hands, equipment, hides or from the gastro-intestinal tract (Gill,1979; Eustace, 1981; Nottingham, 1982; Waites, 1988). It is accepted that when surface microbes are about $2x10^6$ per square centimeter the first off-odour on beef can be detected and slime becomes visible when the numbers of the surface bacteria are $10^8/cm^2$ (Ayres, 1960; Gill, 1985) and yet the ICMSF recommends that the highest limit acceptable for meat is $10^7/g$. This acceptable limit may seem very high but this is per gram not per square centimeter.

5.2 Bacterial numbers

The considerable increase in the counts of the fore-rib both on the nutrient agar (Figure 4.1) medium and on the desoxycholate agar medium (Figure 4.2) are attributable to the fact that this is the carcass position which is

most exposed to handling when the carcasses are pushed along the line (Nortje *et al.*, 1981).

The general log counts of about three log numbers, for the total aerobic counts imply fairly clean and hygienic operations (Eustace, 1981) though not as clean as is the case in some studies which were carried out at a research-linked abattoir not a commercial one (Nortje *et al.*, 1981).

The standard deviations of the average log counts (see Tables 4.3 and 4.6) were seen to be large thus the coefficients of variation were calculated (Table 4.3 and 4.6) and found also to be very large for both media and for all the sampling sites. Norjte *et al.*, (1981) studied the origin of contamination by bacteria on beef carcasses at an abattoir. They reported that there was a significant interaction between the microbiological variation and the type of carcass considered. Probably this interaction may be the same phenomenon as the observed wide variations within my sampling lot. One noticed huge differences between initial counts from one carcass and another. It is unlikely that such differences were attributable to sampling error, one is likely to be convinced that some intrinsic characteristics of the carcasses or their surfaces influences the level of contamination (Kraai *et al.* ,1985).

In one study by Ingram and Roberts, (1976), over a four day period while sampling 25 carcasses per day, on any particular day the range of counts between carcasses was very large, from less than a hundred to many thousands,

even more than 100 000 per cm^2. The reason for such extreme differences is not understood, but isolated occurrences may be responsible. For example, is the occasional bacteriologically dirty carcass the result of the large intestine being punctured by a slaughter-man - as almost inevitably happens on occasion, or could it be a particularly dirty live animal? Similarly the very clean carcass might be the result of a very clean live animal, or perhaps, by rare chance, many operatives simultaneously cleaned their equipment when dealing with that carcass. Whatever the reasons, the existence of this wide variability means that a large number of carcasses must be examined to get a reliable index of the output of a slaughterhouse on any given occasion and samples taken from at least 25 carcasses can only estimate the true situation on the larger number of carcasses comprising a day's throughput at a large abattoir (Ingram and Roberts, 1976).

A large proportion of the bacteria were not re-culturable and our sampling methods are such that they only pick up a small proportion of all the bacteria in a sampling site (Nortje *et al.*, 1982). Our current media are designed only for the culturable bacteria, the non-culturable can only be detected by molecular means. Culturable bacteria represent only a small fraction of the prokaryotic biota. There is evidence of a large reservoir of unculturable bacteria, for example, those discovered solely by their ribosomal RNA sequences (Colwell *et al.*, 1995). There are also the viable but non-cultivable (VBNC) stages of Gram negative bacteria (Xu *et al.*, 1982) as well as the spore

(resting stages) of Gram positive bacteria (Colwell *et al.*, 1995).

In the case of viable but non-culturable bacteria, Gram-negative bacterial cultures, under conditions adverse to growth and cell division, undergo conversion into non-culturable forms. The number of culturable cells decreases with time of incubation (Grimes *et al.*, 1986). Without direct detection techniques, the assumption was that such cultures were dead. However, the use of nucleic acid probes and PCR technology have confirmed the presence of large numbers of metabolizing, but non-dividing cells (Colwell *et al.*, 1995).

To understand meat spoilage one requires knowledge of bacterial growth at chill temperatures. Such an understanding involves knowing which organisms are present on the meat at the start of storage, their origin, the nature of their environment and the effect of their growth on that environment. The most important factors in determining the spoilage flora of fresh meat at chill temperatures are the relative numbers of the initial psychotrophic bacteria and their growth rates at such temperatures (Waites, 1988).

5.3 Bacterial variety

The ratios of the main groups picked up (see Figures 4.3 and 4.4) are in agreement with earlier findings of Empey and Scott, (1939); Ayres, (1960); Gill (1982); Brown and

Baird-Parker, 1982; Nortje, (1987) and Buys *et al.*,(1994). Nottingham (1979) reported that coliforms tend to be more numerous on mutton than on beef, and Dainty (1983) showed that *Pseudomonas* spp. were more common (42 to 60%) while the Moraxella group (including *Moraxella, Acinetobacter* and *Psychrobacter*) contributed 16 to 23% of the flora. *Brochothrix thermosphacta* was much less common on beef (4%) than on lamb (22%) or pork (26%).

The *Pseudomonas* spp. as a group preferentially utilize glucose and strongly suppress degradation of amino acids until glucose is exhausted, but are capable of using most of the naturally occurring amino acids for growth. Growth on the preferentially utilised amino acids is as rapid as on glucose(Newton *et al.*, 1978).

The onset of aerobic spoilage is a function of the metabolic activity of the *Pseudomonas* spp. Initially these bacteria grow at the expense of glucose and do not produce offensive by-products. When the cell numbers at the surface exceed $10^8/cm^2$, the supply of glucose becomes insufficient to meet the bacterial demand and the microbes begin to attack the amino acids. The pH of the meat then rises because of the release of the ammonia. Other undesirable products, such as organic amines (cadaverine, putrescine, isobutylamine) and sulfides (H_2S, methyl sulphide), are formed in relatively smaller quantities, but the intensity of their odours and flavours more than makes up for their low concentrations (Miller, 1973).

Aeromonas spp. As a group together with the Enterobacteriaceae they do not seem to have a big influence on meat but are important as indicator organisms to show the level of hygiene (Gill, 1979). At chill temperatures they grow far slower than strict aerobes, but like *Pseudomonas* spp they produce malodorous by products when utilising amino acids for growth.

Many strains of the *Moraxella/Acinetobacter* group are encapsulated. The group of organisms utilises amino acids as preferred substrates when growing on meat (Juni *et al.*, 1986). They may be expected to be a big role player in meat spoilage. They however do not seem to produce significant amounts of highly offensive metabolic by-products (Gill, 1983). This may be partly because they are sensitive to the low pH of normal meat, hence they are less important than the *Pseudomonas* spp on red meat of normal ultimate pH held at chill temperatures. They are of concern on meat of high pH or when meat is held at ambient temperatures because the effects of pH are then less pronounced (Gill and Newton, 1982).

The proportion of the Micrococcaceae seemed to be on the increase from the beginning of the abattoir operations to the end. Since the primary habitat of these organisms is the mammalian skin it would be understandable to find a steady rise in the relative proportion of the organisms picked up. The carcasses are pushed from one position to the next by direct application of pressure from the hands on the carcass it would there-fore be understandable to find that the relative numbers increase. At the beginning

of storage there may be considerable numbers of the bacteria, with time their numbers are depressed owing to the faster growth of the psychotrophs (Waites, 1988). Micrococci do not seem to be pathogenic to humans. The staphylococci however are known to cause among others food poisoning intoxication.

The facultative anaerobic *Alteromonas* spp an example of which is shown in Figure 4.8, are also important. They do not grow very at pH values below 6.0. They degrade cysteine even in the presence of glucose, and under anaerobic conditions, as in vacuum-packed meat and meat products, H_2S is released with deleterious effects upon meat colour (Gill, 1983). *Alteromonas* spp become important in glycogen-depleted muscle, as in the case is when animals are stressed just before the slaughter, where the pH stays above 6.0 like in D.F.D. meat (Gill, 1983).

The gram positive *Brochothrix* spp are non-spore forming, non-motile, catalase-positive facultative anaerobes. The genus was proposed by Sneath *et al.*, (1976) for some meat spoilage organisms previously designated *Microbacterium thermosphactum*. As stated earlier at the beginning of storage they are usually few in numbers but they then increase in numbers with time.

The major end-products of aerobic metabolism of glucose by *Brochothrix thermosphacta* growing on tryptone-based medium, on a minimal defined medium are acetoin and acetic, isobutyric, isovaleric (3-methylbutyric), and 2-

methylbutyric acid (Dainty *et al.*, 1983, 1985). In a complex medium such as meat, only acetoin and acetic acid are derived from glucose; and isobutyric, isovaleric, and 2-methylbutyric acid are produced from valine, leucine, and isoleucine respectively (Dainty *et al.*, 1983). These compounds or their derivatives ,produce the sweet, sickly odours which characterise the growth of *B. thermosphacta* (Dainty *et al.*, 1985). In mixed culture *Brochothrix thermosphacta* may not be present in large numbers but the products of its metabolism are so potent that their effects out-weigh the depressed relative counts (Dainty *et al.*, 1983).

Unlike proteolytic spoilage bacteria (such as *Pseudomonas*) *B. thermosphacta* is usually found on the surface of meat. Fournaud *et al.* (1980) demonstrated histologically that meat spoilage bacteria, including *B. thermosphacta,* can penetrate the deep muscle mass by following the perimysium or endomysium connective tissue. Contamination of meats almost certainly occurs during slaughter and post-slaughter procedures. The organism has been isolated from hides of cattle (Newton *et al.*, 1978) cattle hair, rumen contents, floors and equipment in slaughter halls (Patterson *et al.*,1975; Talon *et al.*, 1988).

5.4 Conclusion

The overall study was successful in that I was able to get a general view of the types of microorganisms one should expect along a bovine slaughter line. It was not

however, possible for me to be able to get a complete picture reflecting the relative numbers along the line due to the wide variation in the observed counts (mine as well as literature, Ingram *et al.*, 1976; Nortje *et al.*, 1981). The chillers must be maintained at lower temperatures (5°C to 7°C) for them to effectively reduce the bacterial counts on the carcasses before they are taken away to the butcheries or before they are taken away for deboning and further processing.

Recommendations

* From management with the help of the health inspectorate as well as using the part of this study which dealt with bacterial growth on Nutrient agar, down to the workers at the abattoir slaughter line, concerted efforts should be made to teach all the personnel working at the abattoir of the potential microbial effects of organisms found to be prevalent on the carcasses as well as the likelihood of food poisoning. Emphasis should be made of the financial loss of butcheries and supermarkets due to spoilage micro-organisms.

* The management of the abattoir may have to be convinced to join the South African Red Meat Suppliers Association. This way the abattoir will keep a closer look at the sanitation situation of the manufacturing process.

* Funds should be sought for a study involving the vacuum packed meat to evaluate the microbial load of the products there as well as more samples must be taken along the slaughter line so as to reduce the standard deviation and the coefficient of variation.

List of abbreviations used

B.S.E. : Bovine Spongiform Encephalopathy

C.F.C. : Cetrimide Fucidin and Cephaloridin

c.v. : coefficient of variation

D.F.D. : Dark firm and dry

°C : Degrees Celcius

G.M.P. : Good Manufacturing Practice

H_2S : Hydrogen sulphide

H.A.C.C.P. : Hazard Analysis Critical Control Point

H.D.L. : High Density Lipoprotein

I.C.M.S.F. : International Commission of Microbiological Specifications for Foods of the International Association of Microbiological Soceities.

L.T. : Labile Toxin

PCR : Polymerase Chain Reaction

P.S.E. : Pale Soft and Exudative

resp. : respectively

spp. : Species

Std. dev. : Standard deviation

str. : Strain

VBNC : Viable but non culturable

W.H.C. : Water Holding Capacity.

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APPENDIX A

A) Desoxycholate citrate agar (Hynes) (g/l)

Meat extract (Lab-lemco powder)	5,0
Peptone	5,0
Lactose	10,0
Sodium citrate `	8,5
Sodium thiosulphate	5,4
Ferric citrate	1,0
Sodium desoxycholate	5,0
Neutral red	0,02
Agar	12,0

Suspend the above reagents in one litre of distilled water. Bring to the boil with constant stirring until dissolved. Mix well and pour plates immediately. Dry the agar surface before use. The medium is heat sensitive and must not be over-heated or re-heated or autoclaved.

B) Nutrient Agar (g/l)

Meat extract	1,0
Peptone	5,0
Yeast extract	2,0
Sodium chloride	8,0
Agar	15,0

To prepare plates: dissolve the above reagents in one litre of distilled water. With constant stirring heat to the boil. Autoclave at 121°C for 15 minutes. Allow the

medium to cool to about 50°C. When cool dispense about 20 ml into each petri dishes.

To prepare agar slants: dissolve 31g of agar in a litre of distilled water. With constant stirring heat to the boil. When dissolved dispense 9 ml per tube. Cap the tubes, place them on in a rack and autoclave them at 121°C for 15 minutes. After the sterilization stand them at an angle of about 20° to the horizontal on an incline to set.

C) **Pseudomonas agar base (CM559) g/l**

Gelatin peptone	16,0
Casein hydrolysate	10.0
Potassium sulphate	10,0
Magnesium chloride	1,4
Agar	11,0
pH	7,1+- 0,2

C-F-C Supplement Each vial is sufficient to supplement 500.0ml of Pseudomonas agar base (CM559), and contains

Cetrimide	5,0mg
Fucidin	5,0mg
Cephaloridin	25,0mg

Suspend the reagents mentioned above of the agar base CM559, in 500ml of distilled water. Add 5ml of glycerol. Bring to the boil to dissolve completely. Sterilise by

autoclaving at 121°C for 15 minutes . Allow the medium to cool to 50°C and aseptically add the contents of one vial of Pseudomonas CFC supplement (SR103) reconstituted as directed. Mix well and pour 20ml into sterile petri dishes.

Pseudomonas C-F-C selective supplement SR103

Directions Aseptically add 2ml of ethanol/ sterile distilled water (1:1) to one vial and mix gently to dissolve. Avoid frothing. Aseptically add the vial contents to 500ml of sterile Pseudomonas basal medium cooled to ca. 50°C-55°C, prepared from Pseudomonas agar base (CM559) plus 5ml of glycerol. Mix well and pour into sterile petri dishes.

Precautions

The cephaloridin may cause irritation if inhaled or when in contact with the skin.

D) M-R-S agar (deMan, Rogosa and Sharpe) (A selective medium for the enrichment, cultivation and isolation of Lactobacilli from all types of food and pathogenic specimens.)

Special peptone	10,0
Beef extract	5,0
Yeast extract	5,0
Dextrose	20,0
Potassium phosphate	2,0

Tween 80	1,0
tri-Ammonium citrate	2,0
Magnesium sulphate	0,1
Manganese sulphate	0,05
Sodium acetate	5,0
agar	12,0
pH	6,5(+-0,2)

Suspend the above reagents in one litre of distilled water. Allow to stand for 15 minutes. Boil until completely dissolved. Sterilize in the autoclave for 15 minutes at 121°C. Pour into sterile petri dishes.

E) R-F Medium for bacilli (g/l) (Respiratory-Fermentative medium)

Glucose	5,0
Yeast extract	0,2
Ammonium hydrogen Phosphate $(NH_4)_2HPO_4$	1,0
Potassium chloride	0,2
Magnesium sulphate ($MgSo_4.7H_2O$)	0,2
Bromocresol purple	0,008
agar	3,0
pH	7,0

Direction

Heat to the boil to dissolve the ingredients. When dissolved dispense 9ml into each test tubes. Place caps of one colour to one half of all the test tubes. Autoclave at 121°C for approximately 15 minutes.

Allow the tubes to cool to about 50oC and to one half of the total number of the tubes carefully, $1cm^3$ of sterile liquid paraffin was added. The liquid paraffin was sterilized by heating it to about 200°C for two hours in a furnace.

F) Motility medium (Cowan and Steel, 1965).

Gelatin	80g
Peptone	10g
Beef extract	3g
Sodium chloride	5g
Agar	4g
Distilled water	1l

Dissolve the gelatin in water then put the other ingredients in. Heat to dissolve and dispense into test tubes with Craigie tubes. Sterilize

APPENDIX B

1. Acetone alcohol

Acetone	30ml
95% ethanol	70ml

2. Acid alcohol

HCl (37%)	30ml
95% ethanol to 1000ml	

3. Ammonium oxalate crystal violet

Sol. A.	Crystal violet	2,0g
	95% ethanol	20ml.

Sol. B.	Ammonium oxalate	0,8g
	Distilled water	80ml.

Mix A and B.

4. 1.6% Bromocresol purple

Bromocresol purple	16,0g
95% ethanol	500ml
Distilled water	500ml

Dissolve the stain in the alcohol and add the water. Filter.

5. **1:10 Carbol fuchsin**

Add 10ml Ziehl-Neelsen's carbol fuchsin to 90ml distilled water

6. **20% Copper sulphate**

Copper sulphate ($CuSO_4.5H_2O$)	20,0g
Distilled water	100ml.

7. **Crystal Violet**

See ammonium oxalate crystal violet above.

8. **Diluent solution**

Sodium chloride	8,5g
Peptone	1.0
Distilled water	1000ml

9. **Gram's iodine**

Iodine	1,0g

Potassium iodide	2,0g
Distilled water	300ml.

Mix the iodine and potassium iodide in a mortar and grind until fine. Add the water and mix well. Store in tinted bottles.

10. Malachite green

Malachite green	5,0g
H_2O	100ml

11. Safranine

Safranine	0.25g
95% ethanol	10 ml
Distillied water	100ml

Dissolve the safranine in the alcohol, add the water and filter.

12. Ziehl-Neelsen's carbol fuchsin

Basic fuchsin	0,3g
Phenol	5,0g
95% ethanol	10,0ml

Distilled water	95,0ml

Dissolve the stain in the alcohol and the phenol in the water. Mix the two solutions.