THE PREVALENCE OF MEAT-BORNE AND AIRBORNE STAPHYLOCOCCI IN DEBONING AREAS OF LOW- AND HIGH-THROUGHPUT RED MEAT ABATTOIRS

By

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DECLARATION OF INDEPENDENT WORK

I, KARABO SHALE, do hereby declare that this research project submitted to the Central University of Technology for the degree DOCTOR TECHNOLOGIAE: ENVIRONMENTAL HEALTH, is my own work and has not been submitted before to any institution by myself or any other person in fulfillment of the requirements for the attainment of any qualification.

Karabo Shale .... 2004/03/26 ....

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"The task is not so much to see what no one else has yet seen; but to think what nobody has yet thought, about that which everybody sees" – Erwin Schröedinger.

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SUMMARY

THE PREVALENCE OF MEAT-BORNE AND AIRBORNE STAPHYLOCOCCI IN DEBONING AREAS OF LOW- AND HIGH-THROUGHPUT RED MEAT ABATTOIRS

In developing countries members of the staphylococci have consistently been shown to be one of the major micro-organisms responsible for food poisoning outbreaks and red meat is one of the primary vehicles through which staphylococci is transmitted. With countries such as South Africa increasingly looking to export food products to overseas and neighbouring countries, emphasis is placed on the quality of packaging and maintaining of the extrinsic environment during transport. Bioaerosols that are present in the processing rooms of food factories have furthermore been found to contribute to the contamination of the product thereby reducing its quality and shelf-life. In this study, the microbial shelf-life of vacuum-packed beef stored at 5ºC and 18ºC was investigated in order to shed light on the interactions amongst, and predominance of certain microbiota during low temperature, vacuum-packed storage as well as in the event of breaching of the cold-chain. The deboning rooms of selected abattoirs were further investigated for airborne and meat-borne concentrations of staphylococci, their species distribution as well as coagulase types of *Staphylococcus aureus* in particular.

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The initial microbial load played a pivotal role in the patterns of growth at both 5ºC and 18ºC storage temperatures and interactions were noted amongst a number of genera. During storage under vacuum at both the mentioned temperatures the numbers of, amongst others staphylococci, levelled-off as a result of competition for available nutrients and residual oxygen. This suggests that prolonged storage does not necessarily cause the proliferation of only hazardous bacteria in the product. Staphylococcal bioaerosol concentrations varied considerably amongst the abattoirs investigated, ranging from 8 to 3.5 x 10^3 CFU.m^{-3}. *Staphylococcus xylosus* and *Staphylococcus saprophyticus* were found to be the most abundant species in the air of the deboning rooms. *Staphylococcus aureus* coagulase types III and VIII were furthermore found to predominate the isolated coagulase types of airborne *S. aureus*. In the meat itself, the highest staphylococci counts (1.7 X 10^6 CFU.g^{-1}) were enumerated in the high throughput (Grade A) abattoir. Meat-borne counts exceeded the National Guidelines (10^2 CFU.g^{-1}) without exception and at least 50% surpassed the infective dose of 10^5 CFU.g^{-1} determined for *S. aureus*. Staphylococci species were dominated by *S. capitis, S. xylosus, S. auricularis, S. aureus* and *S. intermedius* on red meat. The coagulase types of *S. aureus* were present in all the abattoirs with type V the most dominant and type VI, the least.

The author finally proposes novel approaches to the application of microbiological data as indices of shelf-life using descriptive and inferential means. Although these indices still needs optimization and further investigation, they could pave the way to more complete predictive models for red meat shelf-life and spoilage. Finally, some recommendations are made to the meat industry

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to address the predicament of microbial contamination in abattoir deboning rooms.

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OPSOMMING

DIE VOORKOMS VAN VLEISGEDRAE EN LUGGEDRAE STAFILOKOKKE IN ONTBENINGSAREAS VAN LAE- EN HOëTOEVOER-ROOIVLEISABATTOIRS

In ontwikkelende lande word *Staphylococcus aureus* deurlopend aangetoon as een van die belangrikste mikro-organismes in voedselvergiftigingsuitbrake en rooivleis is een van die mees prominente vervoermiddels waardeur stafilokokke oorgedra word. Omdat lande soos Suid-Afrika toenemend besig is om uitvoer van voedselprodukte na die buiteland en buurlande te ondersoek, word klem toenemend geplaas op die kwaliteit van die verpakkingsmateriaal en die handhawing van die eksintrieke omgewing tydens berging en vervoer. Daarmee saam verminder bio-aërosols in die prosesseringskamers van rooivleisabattoirs die kwaliteit en raklewe van vleisprodukte. In hierdie studie is die mikrobiologiese rakleeftyd van vakuumverpakte beesvleis, gestoor by 5ºC en 18ºC, ondersoek ten einde lig te werp op die interaksies tussen, en dominering van sekere mikrobiota tydens lae temperature, asook in gevalle waar 'n breek in die koueketting voorkom. Verder is die ontbeningsareas van geselekteerde Suid-Afrikaanse rooivleisabattoirs ondersoek vir luggedraagde en vleisgedraagde konsentrasies van stafilokokke, die spesieverspreiding daarvan, asook stollingstipes van *Staphylococcus aureus* in die besonder.

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Die resultate toon aan dat die verskillende genera by bogenoemde twee temperature, totaal verskillend reageer met spesifieke risiko's wat ontstaan vanweë die oorheersing van sekere groepe. Die aanvanklike mikrobe lading het by beide 5°C en 18°C 'n beduidende rol in die groeipatrone van die organisms gespeel en interaksies is waargeneem tussen verskeie genera. Tydens die rakleeftydstudie het verskeie mikrobe groepe, waaronder stafilokokke, 'n afplatting getoon weens kompetisie vir beskikbare voedingstowwe en residuele suurstof. Hierdie waarneming suggereer dat nie net gevaarlike organismes tydens verlengde stoortoestande domineer nie. Stafilokokkale bio-aërosol-konsentrasies verskil aansienlik tussen die abattoirs wat ondersoek is en wissel van 8 tot 3.5 x 10^3 CFU.m^{-3}. Daar is gevind dat *Staphylococcus xylosus* en *Staphylococcus saprophyticus* die oorwegende spesie in die lug van die ontbeningskamers was. Daar is voorts gevind dat *Staphylococcus aureus*-stollingstipes III en IV die geïsoleerde stollingstipes oorheers het. Die hoogste stafilokok-tellings (1.7 x 10^6 CFU.g^{-1}) is waargeneem in die vleis van die hoëtoevoerabattoir (Graad A). Die tellings het die Nasionale Riglyne (10^2 CFU.g^{-1}) sonder uitsondering oorskry en ten minste 50% was meer as die infektiewe dosis van 10^5 CFU.g^{-1}, bepaal vir *S. aureus*. Spesies is gedomineer deur *S. capitis, S. xylosus, S. auricularis, S. aureus* en *S. intermedius*. Die stollings-tipes van *S. aureus* was teenwoordig in al die abattoirs met tipe V die mees dominante en tipe VI die minste.

Die outeur maak laastens verskeie nuwe voorstelle rakende die toepassing van mikrobiologiese data as voorspellings-indekse van rakleeftyd deur gebruik te maak van beskrywende sowel as inferensiële metodes. Hoewel hierdie indekse

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steeds geoptimiseer en verder ondersoek moet word, kan hulle die weg baan vir meer volledige voorspellingsmodelle vir rooivleis kontaminasie en bederf. Laastens word 'n aantal aanbevelings aan die vleisbedryf gemaak ten einde die probleem van mikrobe kontaminasie in abattoir-ontbeningsareas die hok te slaan.

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Legends to Figures

		PAGE
Figure 1.1	Flow diagram for the clean area of a red meat abattoir excluding the condemned area and quartering	5:1
Figure 1.2	A scanning electron micrograph of *Staphylococcus aureus*	10:1
Figure 1.3	The 3-D structure of *Staphylococcal* enterotoxin C2 produced by *Staphylococcus aureus*	11:1
Figure 1.4	The 3-D structure of toxic shock syndrome toxin-1 from *Staphylococcus aureus*	11:1
Figure 1.5	An example of coagulase positive (CPS) and negative staphylococci (CNS)	12:1
Figure 2.1	Growth patterns of various micro-organisms in vacuum-packed red meat stored at 5ºC over a seven-week period	32:1
Figure 2.2	Growth patterns of different micro-organisms in vacuum-packed red meat stored at 18ºC over a 14-day period	32:2
Figure 3.1	Total Viable Counts and *Staphylococcus sp.* counts on red meat from various abattoirs	49:1

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Figure 4.1 The patterns and distribution of Total Viable Counts (TVC) during various sampling intervals of hours and weeks 70:1

Figure 4.2 The patterns and distribution of staphylococci during various sampling intervals of hours and weeks 71:1

Figure 4.3 Average Total Viable Counts (TVC) and Staphylococcus counts from various red meat abattoirs 72:1

Figure 5.1 An example of the application of predictive microbiology to calculate the X or Y coordinates for *Escherichia coli* during vacuum-packed storage at 5ºC 88:1

Figure 5.2 Radar plots showing the Total Viable Counts and *Staphylococcus* sp. in the air from the deboning area of red meat abattoirs over a 5-week period. 89:1

Figure 5.3 Radar plots showing the Total Viable Counts and *Staphylococcus* sp. in the meat from the deboning area of red meat abattoirs over a 5-week period 89:2

Figure 5.4 Radar plots showing the Total Viable Counts in the meat and air in the deboning area of red meat abattoirs over a 5-week period 90:2

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Figure 5.5 Radar plots showing the *Staphylococcus* sp. counts in the meat and air in the deboning area of red meat abattoirs over a 5-week period 90:3

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Legends to Tables

		PAGE
Table 1.1	Classification of red meat abattoirs in South Africa (Van Zyl, 1998)	4:1
Table 1.2	New grading system as stipulated in the draft regulation on red meat by the National Department of Agriculture, RSA (2003)	4:2
Table 2.1.	Correlations amongst various microbiota at 5ºC vacuum-packed storage	34:1
Table 2.2	Correlations amongst various microbiota at 18ºC vacuum-storage	35:1
Table 3.1	Staphylococcal species on red meat from various abattoirs	51:1
Table 3.2	*Staphylococcus aureus* coagulase types on red meat isolated from various abattoirs	53:1
Table 4.1	Staphylococcal species found in the air of deboning areas of various abattoirs	73:1

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Table 4.2 Coagulase types of *Staphylococcus aureus* from the air of deboning areas of various abattoirs 75:1

Table 5.1 Mathematical equations representing patterns of microbial growth during storage temperatures of vacuum-packed red meat at 5ºC 87:1

Table 5.2 Mathematical equations representing patterns of microbial growth during storage temperatures of vacuum-packed red meat at 18ºC 87:2

Table 5.3 Statistical correlations between aerobic plate counts and *Staphylococcus* sp. from red meat and the air per abattoir 90:1

Table 5.4 Statistical correlations between airborne and meat-borne aerobic plate counts and *Staphylococcus* sp. per abattoir 90:4

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TABLE OF CONTENTS

PAGE

List of Figures viii

List of Tables xi

Chapter 1: Introduction 1

1.1 Introductory remarks 2

1.2 Increasing awareness of risks associated with red meat 6

1.3 Meat as a medium for microbial growth 8

1.4 Outline of the *Staphylococcus* genus 10

1.5 Rationale 13

1.6 References 16

Chapter 2: The influence of storage environment on the microbial proliferations in vacuum-packed red meat 25

2.1 Abstract 26

2.2 Introduction 27

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2.3 Materials and methods 29

2.4 Results and discussion 32

2.5 References 37

Chapter 3: The distribution of *Staphylococcus* sp. on bovine meat from abattoir deboning rooms 42

3.1 Abstract 43

3.2 Introduction 44

3.3 Materials and methods 46

3.4 Results and discussion 49

3.5 References 56

Chapter 4: The distribution of staphylococci in bioaerosols from red meat abattoirs 63

4.1 Abstract 64

4.2 Introduction 65

4.3 Materials and methods 67

4.4 Results and discussion 70

4.5 References 78

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2.3 Materials and methods 29

2.4 Results and discussion 32

2.5 References 37

Chapter 3: The distribution of *Staphylococcus* sp. on bovine meat from abattoir deboning rooms 42

3.1 Abstract 43

3.2 Introduction 44

3.3 Materials and methods 46

3.4 Results and discussion 49

3.5 References 56

Chapter 4: The distribution of staphylococci in bioaerosols from red meat abattoirs 63

4.1 Abstract 64

4.2 Introduction 65

4.3 Materials and methods 67

4.4 Results and discussion 70

4.5 References 78

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Chapter 5: Descriptive and inferential indices of red meat spoilage and contamination 85

5.1 Mathematical models of vacuum-packed red meat spoilage 86

5.2 Application of predictive indices 88

5.3 The contribution of staphylococci and bioaerosols as contaminants of red meat in the deboning area 89

5.4 References 92

Chapter 6: Conclusions 93

6.1 Key observations 95

6.2 Recommendations to industry 96

6.3 Future research 99

6.4 References 101

Appendixes 102

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CHAPTER 1

INTRODUCTION

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1.1 Introductory remarks

References to meat in South Africa are to be found from as early as the 15th century, when the Portuguese exchanged copper ornaments with the Hottentots for fresh meat during their trips to the East Indies. Today the animal production industry is the largest agricultural industry in South Africa and contributes to more than 40% of agricultural production, with the average per person consumption for beef at 12.4 kg per year (South African Meat Industry Companies (SAMIC), 2002). In 2001, cattle numbers in commercial areas amounted to 8,7 million herds, with 4,7 million in developing areas. With a well-established feedlot sector, South African cattle feedlots have a standing capacity of 320 000 animals and around 70% of the cattle in commercial sectors are slaughtered annually at registered abattoirs (SAMIC, 2002).

An abattoir is commonly known as a "food factory with the primary aim of producing a healthy, wholesome and clean meat product which is safe for consumption" (Van Zyl, 1995; Meat Inspectors Manual for Red Meat (MIMRM), 2000). An abattoir, according to Section 1 of the Abattoir Hygiene Act of 1992, is furthermore defined as "any place where animals are slaughtered or are intended to be slaughtered, and includes all facilities which normally appertain or are attached to such place, whether or not such facilities are situated at the same place as such place" (Abattoir Hygiene Act of 1992). In days gone by, the term used for an abattoir was "slaughter poles," where vertical poles were joined by a

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horizontal crossbar on which the animals could be hoisted. In other cases a branch of a tree instead of poles was used for slaughtering (Van Zyl, 1995). In the late sixties, the South African abattoir industry found itself in dire straits because of inadequate facilities for handling the available stock. It was thus concluded that rationalisation would be the solution with the main objective to ensure that abattoirs either be improved or closed down.

In recent times, as the population burgeoned and as increased awareness of hygiene and food-borne illnesses grew, modern abattoirs came into use. These coincided with political changes taking place over the previous decade that led to developments in rural areas, empowering such communities. These developments have also improved the general financial status of previously disadvantaged individuals and have led to a demand for better quality food (Derbyshire, 2002). Van Zyl (1995) reports that with regard to modern abattoirs, the role players are the producers, marketing agents, the abattoir owner and management, workers, suppliers and consumers. National bodies that control the well-being and structures of red meat abattoirs include the Red Meat Abattoir Association, the Meat Board and the Agricultural Research Council. All of these have a role to play either directly or indirectly in ensuring that the product is healthy, wholesome and of good quality (Van Zyl, 1995).

After deregulation of the meat industry in 1993, population growth and the demand for red meat has led to an increased number of registered abattoirs

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coming into business. In the year 2002, the total slaughter capacity was estimated at 16 500 slaughter units per day in registered abattoirs (SAMIC, 2002). This production is carried out in high and low throughput abattoirs with various grading systems as shown in Table 1.1. The purpose of grading has been to facilitate the enforcement of the Act as well as to rationalise the abattoir industry. The first norm of grading, which became valid in 1970, was based on the throughput of slaughter stock on a daily and weekly basis.

Based on the changes and challenges faced by the meat industry in South Africa, the Department of Agriculture has recently drafted legislation that proposes a new grading system (Table 1.2). According to this system, future abattoirs will be graded according to the proposed draft regulations that suggests classification based on three throughput categories. The future grading system also addresses deboning rooms and stipulates that the temperature at which deboning should be done should not exceed 12°C. In the draft legislation, some abattoirs will debone the carcasses (cutting plants) while others will not, although both types of abattoirs will have the same throughput (Republic of South Africa (RSA) Department of Health, 2003).

Because an abattoir represents a type of food factory, it has to be designed in such a way as to reach optimal effectiveness. The layout of the premises should be designed in such a manner that the production process moves in one direction without any cross-flow of products which in turn could adversely affect

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Table 1.1 Classification of red meat abattoirs in South Africa (Van Zyl, 1998)

Abattoir(s)	South African Grading	Throughput (slaughter units* per day)
A	Grade A	>100
B	Grade B	51-100
C	Grade C	16-50
D	Grade D	9-15
E	Grade E	<9

*1 slaughter unit: 1 cattle (bovine), 1 horse, 15 pigs, 15 sheep

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Table 1.2 New grading system as stipulated in the draft regulations on red meat by the RSA, National Department of Agriculture (2003)

New grading	Old Grading	Throughput (slaughter units* per day)
High throughput	Grade A, B, C	Based on capacity of the abattoir (and decided by the provincial executive officer)
Low throughput	Grade D	<20
Rural	Grade E	<2
Own and religious consumption		1 per 14 days

*1 slaughter unit: 1 cattle (bovine), 1 horse, 15 pigs, 15 sheep

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the hygiene of the product (Van Zyl, 1995; MIMRM, 2000). In present designs, live slaughter animals are received in the "dirty" area (reception, offloading, ante-mortem, stunning, hoisting and bleeding) of the abattoir and meat is out loaded from the "clean" side. The "clean" area of the abattoir is illustrated in Figure 1.1 and the dotted boxes represent the focus points for the present study. The carcasses are normally chilled at 2ºC to reduce possible microbial proliferation introduced during slaughtering and processing and to further preserve the attractiveness and freshness of the meat (Gill, 1996; Ware *et al.*, 1999; Retuer, 2000). In high throughput abattoirs the chilled carcasses are normally deboned at 4ºC. During this process the carcasses undergo extensive handling and there is a stepwise increase of microbial counts on the carcasses and/or retail cuts (Nortjé *et al.*, 1990; van Zyl, 1995; Nel *et al.*, 2004). Once the various retail cuts have been removed from the carcass, they are vacuum-packed and transported at temperatures <10ºC, while the remaining cuts are stored frozen at -18ºC for later distribution (Nel *et al.*, 2004).

To circumvent microbial proliferation at low storage temperatures, meat processors use vacuum-packing which provides improved shelf-life during long term storage as well as inter-continental transport (Buzby *et al.*, 1990; Lee and Yoon, 2001). However, aerobic micro-organisms utilise the residual oxygen trapped during vacuum-packing and are able to multiply during the first few days of storage (Aberle *et al.*, 2001). Under these conditions, metmyoglobin is formed from the engrossed oxygen, resulting in the lowering of pH and the production of

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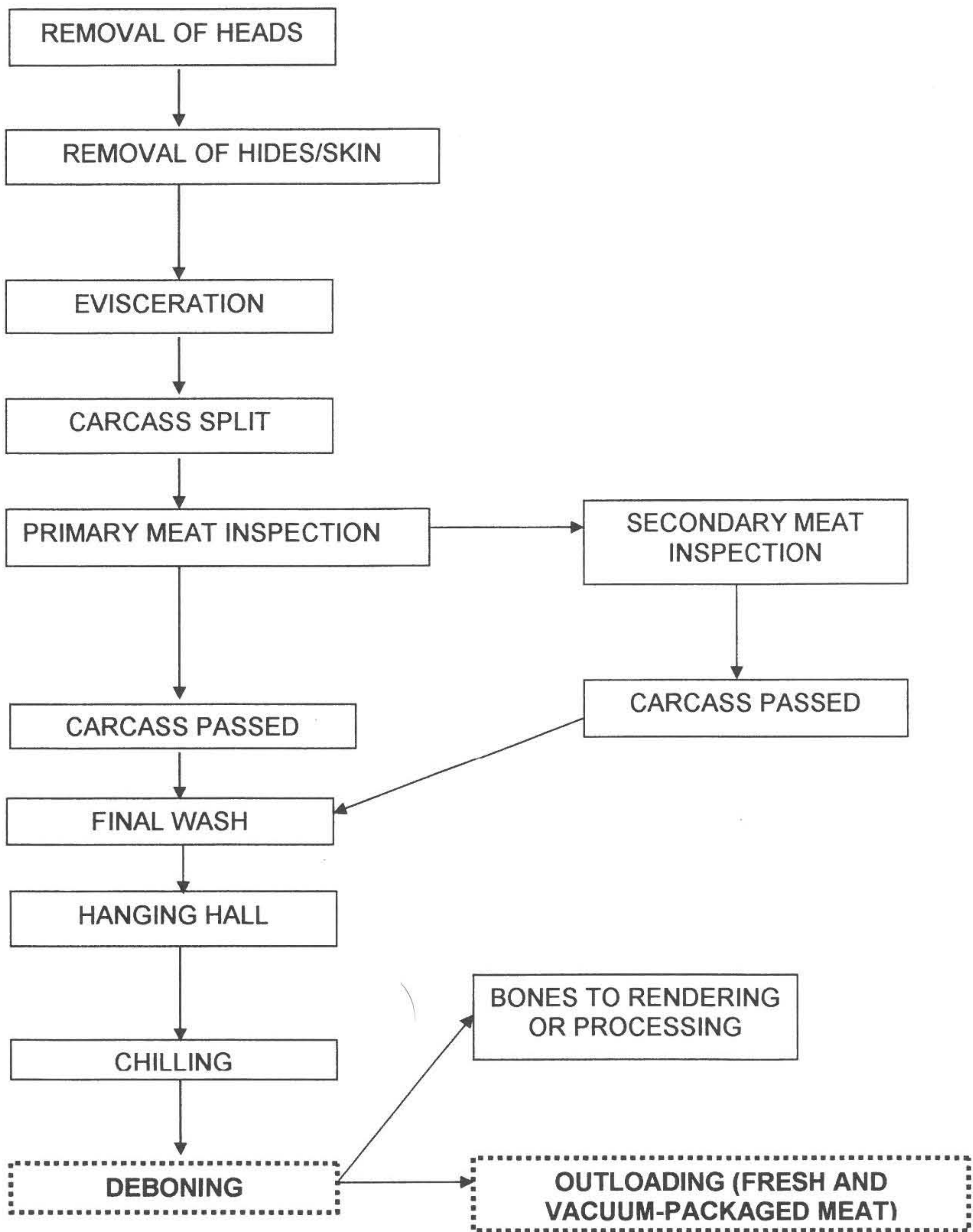

Figure 1.1 Flow diagram for the clean area of a red meat abattoir excluding the condemned area and quartering (MIMRM, 2000)

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carbon dioxide. Although low oxygen levels and pH generally culminate in a low microbial growth and longer shelf-life, facultative anaerobic bacteria use these conditions to multiply and produce lactic acid (Aberle *et al*., 2001).

1.2 Increasing awareness of risks associated with red meat

The red meat industry has been a major force in changing the role of individuals involved in the supply chain of red meat. Red meat industries have explored strategic ways to meet consumer requirements while complying with local food safety regulations and guidelines (Fearne *et al*., 2001). One of the strategies has been the implementation of quality assurance programmes - a strategic management function concerned with the establishment of policies, standards and systems for maintaining quality. This has been necessitated by the fact that there have been cases of food-borne disease resulting from the consumption of raw or undercooked meat, from poor slaughtering techniques, poor inspection of carcasses and viscera, and from unhygienic conditions in abattoirs. The consequences are well stated in the following comment: "In some western countries, the incidence of food-borne diseases may be second only to that of the common cold" (Council for Scientific and Industrial Research (CSIR), 2002). In developing countries the situation regarding food safety and security is a cause for even greater concern, because in these countries at least six children die from diarrhoea every minute, four of them due to contaminated food (CSIR, 2002).

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Between 1988 and 1992, 70% of all food-borne disease outbreaks in the USA were attributed to bacterial pathogens and beef was one of the major sources of transmission (Scanga *et al.*, 2000). Berends and van Knapen (1999) report that meat inspection procedures are no longer adequate for the protection of public health. As a result, risk assessment should be introduced, even though this is a new science and lacks much of the necessary data. The Australian meat industry commissioned its first baseline study of microbiological quality of beef and sheep carcasses between 1993 and 1994. This survey led to the implementation of comprehensive hazard analysis and critical control point quality assurance plans in all slaughter and the boning facilities. All this was done to prevent excessive microbial loads on meat carcasses (Sumner *et al.*, 2002).

In other areas of the world like the USA, where food safety has become a major concern, research has indicated that beef packers are spending enormous amounts of time and resources on contamination prevention and are, to a large extent, succeeding in efforts to prevent microbiological contamination and in minimising the presence of pathogens (Kain *et al.*, 1999). A baseline survey was furthermore conducted by the Food Safety and Inspection Services (FSIS) between 1992 and 1993 in the USA, which found that sampling procedures, handling and storage systematically influenced the microbiological status of beef (Ware *et al.*, 1999). It is estimated that in the USA alone 10 million people will become sick from eating spoilt and contaminated foods each year, while in Europe a similar situation is anticipated with 300 out of 1000 people expected to

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suffer from food poisoning (CSIR, 2002). In South Africa, however, no formal records have been kept prior to 1989. According to a survey done in South Africa on food poisoning incidents, there were 1, 81, 92 and 334 cases in 1991, 1992, 1994 and 1993 respectively with only one reported fatality in 1994. Given the population of 38 million, it is obvious that the surveillance system is completely ineffective (CSIR, 2002).

1.3 Meat as a medium for microbial growth

The micro-organisms present in food products are dependent on the microbiological quality of raw materials, hygienic conditions during production and storage conditions of the product after processing. In addition, both intrinsic and extrinsic conditions determine the prevalence and multiplication of micro-organisms on the product (Gill and Jones, 1999). Keeping in mind that red meat originate from warm blooded animals, this suggests that their microbiota consist heterogenously of mesophilic bacteria. Some of these bacteria include pathogenic species from the animal itself, while from the environment bacterial species are introduced during slaughter and processing of raw products Gill and Jones, 1999; Jordan *et al.*, 1999).

Red meat has an a_w >0.99 and a pH range of 5-7, which is an optimal combination for microbial growth and fresh meat is a rich medium, relatively strongly buffered (Jay, 2000). Because of the favourable composition of red meat

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for microbial growth, the presence of micro-organisms in the meat raises serious concerns regarding its safety and quality as well as its associated products. Consequently, a great deal of research has taken place over the past few years on microbial populations associated with meat and their effect on meat quality and safety (Nortjé *et al.*, 1990).

It is generally accepted that during slaughtering the animal is regarded as free from bacteria, and extrinsic sources of bacterial contamination are usually small amidst good hygiene practices. However, the conditions under which the animals are slaughtered and processed determine the microbial populations on the carcasses (Nortjé *et al.*, 1990; Forsythe, 2000; Borch and Arinder, 2002). Unavoidable reservoirs of micro-organisms include the hide, the fleece and the gut contents. Most of these micro-organisms are of faecal origin, normal microbes of the skin (staphylococci, micrococci, pseudomonads, yeasts and molds) and organisms from water and soil (Nortjé *et al.*, 1990; Eisel *et al.,* 1997; McEvoy *et al.*, 2000). In addition, contamination of the carcasses can be introduced through food handler's hands, clothes and knives, conveyer belts and other processing equipment and surfaces in the deboning room, although the exact effects of these sources are only infrequently touched on in literature (Eisel *et al.*, 1997; Ammor *et al.*, 2004; Nel *et al.*, 2004).

Microbial populations associated with fresh red meat include, amongst others, *Pseudomonas* sp., *Acetobacter* sp., *Enterobacter* sp., *Brochothrix* sp., *Moraxella*

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sp., *Lactobacillus* sp., *Flavobacterium* sp., *Vibrio* sp., *Aeromonas* sp. and *Arthrobacter* sp. with *Pseudomonas* sp. usually dominating at low temperatures (Forsythe, 2000; Insausti *et al.*, 2001). Other micro-organisms that can also be found on fresh red meat are members of the *Enterobacteriaceae*, *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Listeria monocytogenes* and *Salmonella* sp. (Nel *et al.*, 2004). When the meat is contaminated by these microbes, it might undergo discolouration, pigment decomposition, deterioration of flavour and development of off-odours due to anaerobic bacteria (Lee and Yoon, 2001).

1.4 Outline of the *Staphylococcus* genus

The *Staphylococcus* genus consists of 38 species (Edwards *et al.*, 2001; Elliot *et al.*, 2003). Staphylococci are Gram-positive, spherically-shaped bacteria (cocci) that can be divided on more than one plane to form irregular clusters of cells (Figure 1.2). Staphylococci are chemo-organotrophs with their metabolism either respiratory or both respiratory and fermentative. All members are known to be catalase-positive and produce an acid not easily detected by standard methods during the metabolism of glucose (Elliot *et al.*, 2003).

Staphylococci are widely distributed in nature, are ubiquitous and are facultative parasites of man and animals while they survive and propagate in the environment as saprophytes. Man is the primary source of staphylococci as well

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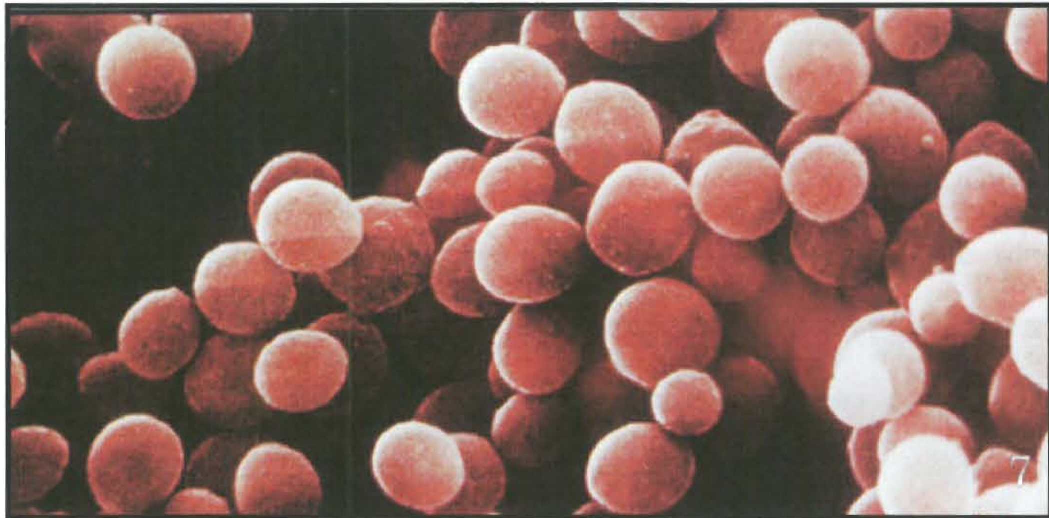

Figure 1.2 A scanning electron micrograph of *Staphylococcus aureus* (Todar, 2002)

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as the principal vector for their transmission as they occur naturally on the skin, in skin glands and in mucous membranes of warm-blooded animals (Wieser and Busse, 2000; Nagase *et al.*, 2002). Animals also harbour staphylococci and serve as their reservoirs. The importance of staphylococci in the food sector lies in the fact that they are normally shed from human and animals, thus continually contaminating the environment (Uche and Agbo 1985; Desmarchelier *et al.*, 1999; Benito *et al.*, 2000; Mosupye and Von Holy, 2000; Miwa *et al.*, 2000; Atanassova *et al.*, 2001; Hein *et al.*, 2001; Nagase *et al.*, 2002; Elliot *et al.*, 2003; Palomares *et al.*, 2003; Nel *et al.*, 2004).

Staphylococci can assume various roles in nature: 1) they are found free-living in the environment; 2) some strains, due to their capability to cause infections, are a major root of morbidity and death, and 3) some strains cause food-borne intoxications. It is, however, difficult to discuss the sources of staphylococci without considering the sources of the various strains because there is no evident pattern according to which organisms from certain sectors of the environment are destined to be commensals while others will eventually cause diseases. Furthermore, there is no apparent relationship among strains that cause infections and those that cause intoxications. Foods are likely to become contaminated by strains of organisms from almost any source.

The toxins (Figures 1.3 and 1.4) produced by staphylococci can lead to food-borne illnesses once ingested (Todar, 2002). These toxins are heat-stable

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Figure 1.3 The 3-D structure of *Staphylococcal* enterotoxin C2 produced by *Staphylococcus aureus*

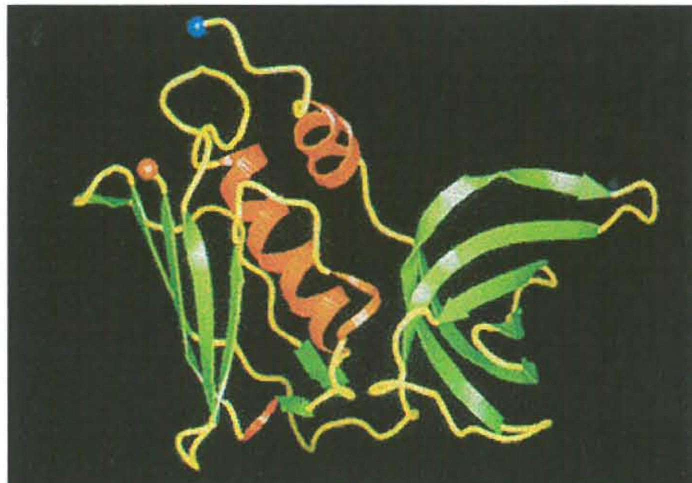

Figure 1.4 The 3-D structure of toxic shock syndrome toxin-1 from *Staphylococcus aureus*

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enterotoxins that attack the gastro-enteric tract causing severe vomiting, nausea, abdominal cramps and prostration with diarrhoea (Reed, 1993; Elliot *et al*., 2003). Symptoms can appear within 30 min to 7 hours after ingestion of the toxin, although on average 2 to 4 hours is the time that the toxin takes to have an effect. The duration of illness is from 1 to 2 days (Reed, 1993).

Staphylococci comprise of coagulase positive and negative members (Figure 1.5) with *Staphylococcus aureus,* belonging to the coagulase-positive staphylococci (CPS), the most common enterotoxigenic staphylococcal species causing food-borne diseases (Ieven *et al*., 1995; Desmarchelier *et al*., 1999). Coagulase-negative staphylococci are often found among the normal biota of human skin and mucous membranes and are likely to be pathogenic under favourable conditions. Nosocomial infections caused by these bacteria are becoming increasingly problematic in many countries. The coagulase-negative staphylococci do not produce plasma coagulase and are differentiated from others based on their resistance to certain antibiotics as a result of being nosocomial (Eiff *et al*., 2002). *Staphylococcus saprophyticus* subsp *saprophyticus, S. cohnii* subsp. *conhii, S. sciuri* and *S. xylosus* are known to be novobiocin-resistant. On the other hand, coagulase-negative staphylococci isolated from human and animal specimens (*S. epidermidis, S. hamolyticus, S. auriularis, S. capitis* subsp. *capitis, S. capatis* subsp. *urealyticus, S. caprae, S. hominis, S. ludgunensis, S. pasteuri, S. saccharolyticus, S. schleiferi subsp*

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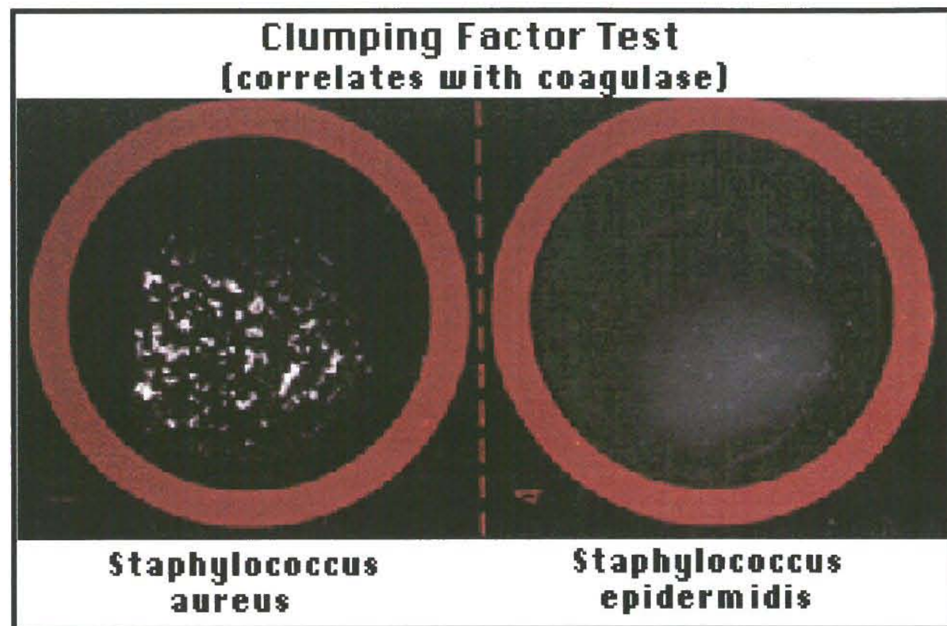

Figure 1.5 An example of coagulase-positive (CPS) and negative staphylococci (CNS)

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schleiferi, S. simulans and *S. warneri*) are all novobiocin-susceptible (Eiff *et al.*, 2001).

Staphylococcus aureus has been known to be involved in food-associated illnesses since 1884 when the first recorded outbreak linked to Cheddar cheese was documented. In 1914 *S. aureus* was recognised as causing illnesses in individuals consuming mastitis-infected cow's milk (Sandel and McKillip, 2002). This species is the most notorious of all staphylococci and referred to by almost all legislative documents on food (Edwards *et al.*, 2001; Elliot *et al.*, 2003; Sandel and McKillip, 2004). *Staphylococcus aureus* is considered a commensal because serious infections can result from opportunistic strains under appropriate conditions. Some of the conditions that may predispose individuals to serious infections include: 1) skin injuries; 2) infection with viruses; 3) presence of foreign bodies; and 4) chronic underlying diseases. *S. aureus* can cause infections from minor skin infections to life-threatening systemic illnesses (Sandel and McKillip, 2002; Elliot *et al.*, 2003).

1.5 Rationale

Well-recognised pathogens such as staphylococci are increasing in prevalence and some strains are becoming increasingly resistant to anti-microbial agents and detergents. Consequently, addressing emerging food-borne diseases will require more sensitive and timely surveillance, enhanced methods of laboratory

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identification, typing, and identification of effective prevention and control strategies.

Given the fact that staphylococci occur almost everywhere, deboning rooms of red meat abattoirs are prominent areas for encountering this bacteria. Red meat abattoirs use deboning rooms for removal of retail cuts from the carcasses and during this stage the product undergoes extensive handling and exposure to surfaces and utensils (Gill *et al*., 1998; Gill and Jones, 1999; Nel *et al*., 2004). As a result, deboning rooms are prone to several factors which can contribute to the distribution of staphylococci such as: 1) conveyer systems and cutting tables, 2) movement and actions of food handlers, especially through breathing, speaking, sneezing, or coughing, 3) water drains and splashing formed during high-pressure water washing of the floor and 4) the ventilation system (Ren and Frank, 1992). Concerning the presence of *Staphylococcus aureus* in red meat, South African legislation stipulates a maximum of 10^2 CFU.g^{-1}, as 10^6-10^8 CFU of this organism per gram of meat is a sufficient level to produce large quantities of toxins (Meat Inspectors Manual, 2000; Hein *et al*., 2001; SAMIC, 2002).

The aims of this study are therefore:

- to evaluate the microbial shelf-life at 5ºC and 18ºC of vacuum-packed red meat from a prominent South African high throughput abattoir and to

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CHAPTER 2

The influence of storage environment on the microbial proliferation in vacuum-packed red meat

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2.1 Abstract

Microbial proliferation on red meat carcasses stored at low temperatures is known to occur. This proliferation is to a large extent dependent on the types of organisms present as well as on the initial microbial load. In this study, the microbial shelf-life of vacuum-packed beef stored at 5ºC and 18ºC was investigated. This was done to investigate the proliferation of the various microbiota at initial storage temperatures as well as to simulate conditions where a breach in the cold-chain might occur. Throughout the 5ºC storage period, the numbers of *Enterococcus* sp. and *Proteus* sp. decreased whereas those of the remaining micro-organisms increased at a relatively slow pace. Lactose fermenting *Enterobacteriaceae*, *Pseudomonas* sp., *Enterococcus* sp. and coliform counts decreased after ten days of incubation at 18ºC. Strong positive correlations were noted between a number of genera at both storage temperatures. The results show that at these two temperatures, the various genera reacted totally differently with specific hazards originating from the predominance of certain groups. The initial microbial load played a pivotal role in the patterns of growth at both 5ºC and 18ºC.

Keywords: Shelf-life, red meat, vacuum-packing.

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2.2 Introduction

Vacuum-packing is employed by the majority of meat producers to circumvent microbial proliferation at low temperatures, thus providing an improved shelf-life during long-term storage and inter-continental transport (Lee and Yoon, 2001; Blixt and Borch, 2002). In South Africa, high throughput and export abattoirs utilise vacuum-packing to extend the shelf-life of their products for transportation over considerable distances, since the final destination of these products often includes neighbouring countries. Although the monitoring of low temperatures is considered to be of the utmost importance during transportation, the accidental breaking of the cold-chain over long distances and as a result of considerable handling, cannot be ruled out.

On several occasions over the last two decades, regulatory authorities and scientists have voiced concerns regarding the bacterial quality of red meat stored for prolonged periods at poorly controlled temperatures (Nortjé *et al.*,1990; Schlundt, 2002). The growth of pathogenic bacteria, even in vacuum-packed red meat, does occur (Buzby *et al.*, 1995; Gill, 1996; Gray *et al.*, 1996; Kennedy *et al.*, 2000; Aberle *et al.*, 2001; Insausti *et al.*, 2001; Lee and Yoon, 2001). Consequent reports on the source and composition of bacterial populations on fresh and stored red meat have emphasised the importance of good slaughtering practices and proper post-processing storage and packaging (Nortjé *et al.*, 1990; Collins *et al.*, 1995; Forsythe, 2000; Insausti *et al.*, 2001; Borch and Arinder

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2002). Several microbial reservoirs on animals were identified that amongst others included the hide, fleece and gut contents that harbour organisms of faecal, water and soil origin, (Nortjé *et al.*, 1990; Eisel *et al.*, 1997; McEvoy *et al.*, 2000). Eisel *et al.* (1997) furthermore report that micro-organisms are introduced by meat handler's hands, clothes, knives, conveyer belts, general processing equipment and surfaces in areas such as the deboning room.

According to Buys *et al.* (2000) the mode of packaging determines the shelf-life of fresh red meat as well as its attractiveness. Shelf-life studies have revealed that the initial microbial load and the prevailing storage conditions affect the microbial interaction and proliferation patterns during the storage of red meat to a considerable extent (Nortjé *et al.*, 1990; Gill, 1996; Ware *et al.*, 1999). When retail cuts are vacuum-packed and stored at temperatures <10ºC for instance, aerobic micro-organisms utilise the trapped oxygen during the first few days. This microbial proliferation is also concomitant with and dependent upon chemical changes in the meat (Buzby *et al.*, 1995; Aberle *et al.*, 2001; Insausti *et al.*, 2001; Lee and Yoon, 2001).

Microbial genera associated with fresh red meat include *Acetobacter* sp., *Enterobacter* sp., *Brochothrix* sp., *Moraxella* sp., *Lactobacillus* sp., *Flavobacterium* sp., *Vibrio* sp., *Aeromonas* sp., *Arthrobacter* sp., and *Pseudomonas* sp. (Forsythe, 2000 and Insausti *et al.*, 2001). Besides the mentioned genera, members from the genera *Bacillus, Staphylococcus,*

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Escherichia, Listeria, and *Salmonella* are also periodically found on fresh red meat (Nel *et al.*, 2004). In the event of contamination, growth of these organisms on red meat might result in discoloration, pigment decomposition, deterioration of flavour, and the development of off-odours (Gill, 1996; Killcast and Subramanian, 2000; Lee and Yoon, 2001). The systemic deterioration of meat thus renders it unattractive and usually unfit for human consumption for both aesthetic and safety reasons (Gill, 1996; Gray *et al.*, 1996; Kennedy *et al.*, 2000).

The aim of this study is to evaluate the microbial shelf-life at 5°C and 18°C of vacuum-packed red meat (beef) from a prominent South African high throughput abattoir. The cattle for slaughtering originated from a feedlot. The study further evaluates the growth patterns of selected food-borne microbiota in the mentioned product.

2.3 Materials and methods

2.3.1 Sampling protocol

Vacuum-packed red meat samples were collected from a high throughput (Grade A) abattoir situated in central South Africa (Free State Province). Eight kilograms of fresh red meat sample of beef carcass (hind quarter) was vacuum-packed using a Cryovac VC14 (Sealed Air Corporation) and transported at 0°C to the laboratory. This sample was aseptically sub-divided into 12 (+/-700g) pieces and

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again vacuum-packed. Zero hour sample was analysed immediately while the remaining samples were subdivided and 6 held at 5ºC and 6 at 18ºC respectively. Quantification of selected micro-organisms was done at weekly and 2-day intervals for samples stored at 5ºC and 18ºC respectively. For statistical purposes, Spearman's correlation matrix from Microsoft Excel 2000 was used.

2.3.2 Sample preparation and bacterial analysis

Ten gram of the samples were blended in 90ml sterile peptone buffered water (Biolab-SA) using a stomacher (Seward 400) to achieve an initial 1:9 meat dilution. Further dilutions were prepared in peptone water and 0.1ml aliquots plated onto the media described below using the spread-plate method (Herbert, 1990). All analyses were done at least in triplicate.

2.3.2.1 Total Viable Counts and *Enterobacteriaceae*

For the enumeration of Total Viable Counts, Plate Count Agar (PCA, Merck-SA) plates were incubated at 25ºC for 72 hours (Voster *et al.*, 1994). Violet Red Bile Glucose Agar (VRBGA, Merck-SA) was used for enumeration of *Enterobacteriaceae* and incubated at 32ºC for 48 hours (Hayes, 1985).

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2.3.2.2 *Staphylococcus* sp., *Pseudomonas* sp. and *Enterococcus* sp.

Baird-Parker Agar (Biolab, SA) plates were used for the quantification of *Staphylococcus* sp. after incubation at 35°C for 48 hours. Cetramide agar plates (CA, Merck-SA) were incubated at 25°C for 24 hours for the enumeration of *Pseudomonas* sp. (typical colonies were fluorescent under ultra violet light at 366nm) (Goto and Enomoto, 1970). *Enterococcus* Selective Agar (ESA, Merck-SA) plates were used for enumeration of enterococci and incubated at 35°C for 48 hours.

2.3.2.3 *Escherichia coli*, *Salmonella* sp., *Citrobacter* sp. *Proteus* sp. and Total Coliforms

Chromocult Coliform Agar (CCA, Merck-SA) plates were used for enumeration of *E. coli*, *Citrobacter* sp., total coliforms and *Salmonella* sp. (Manafi and Kneifel, 1989). Brilliant Green Agar (Brilliant green agar, Anatech-SA; Osborn and Stokes, 1955) together with Xylose Lysine Deoxycholate (XLD, Anatech-SA) and *Salmonella Shigella* (SSA, Anatech-SA) plates were used in combination with CCA for presumptive enumeration of *Salmonella* sp. BGA was furthermore used for the enumeration of presumptive *Proteus* sp. All plates were incubated at 35°C for 48 hours.

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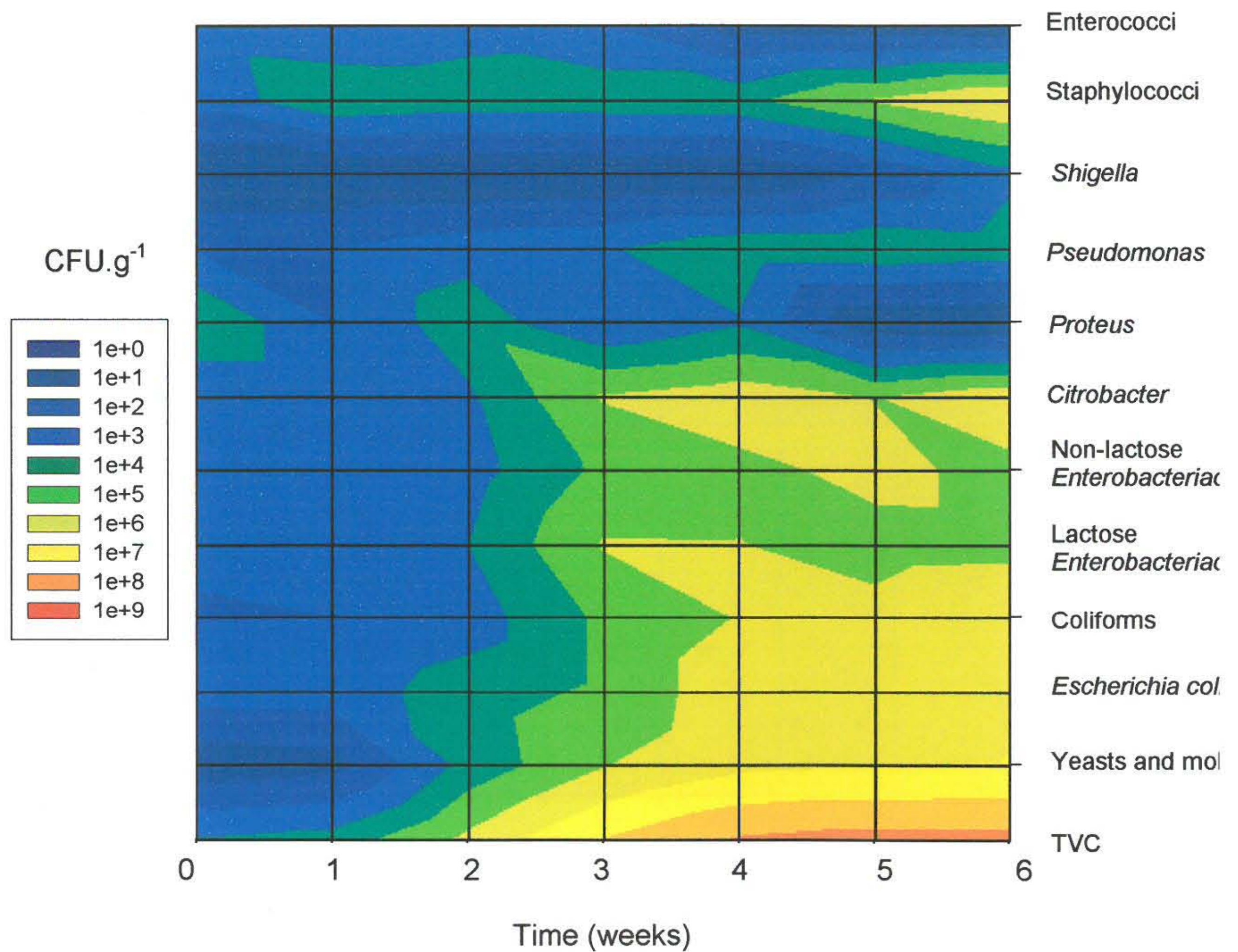

Figure 2.1 Growth patterns of various micro-organisms in vacuum-packed red meat stored at 5ºC over a seven-week period.

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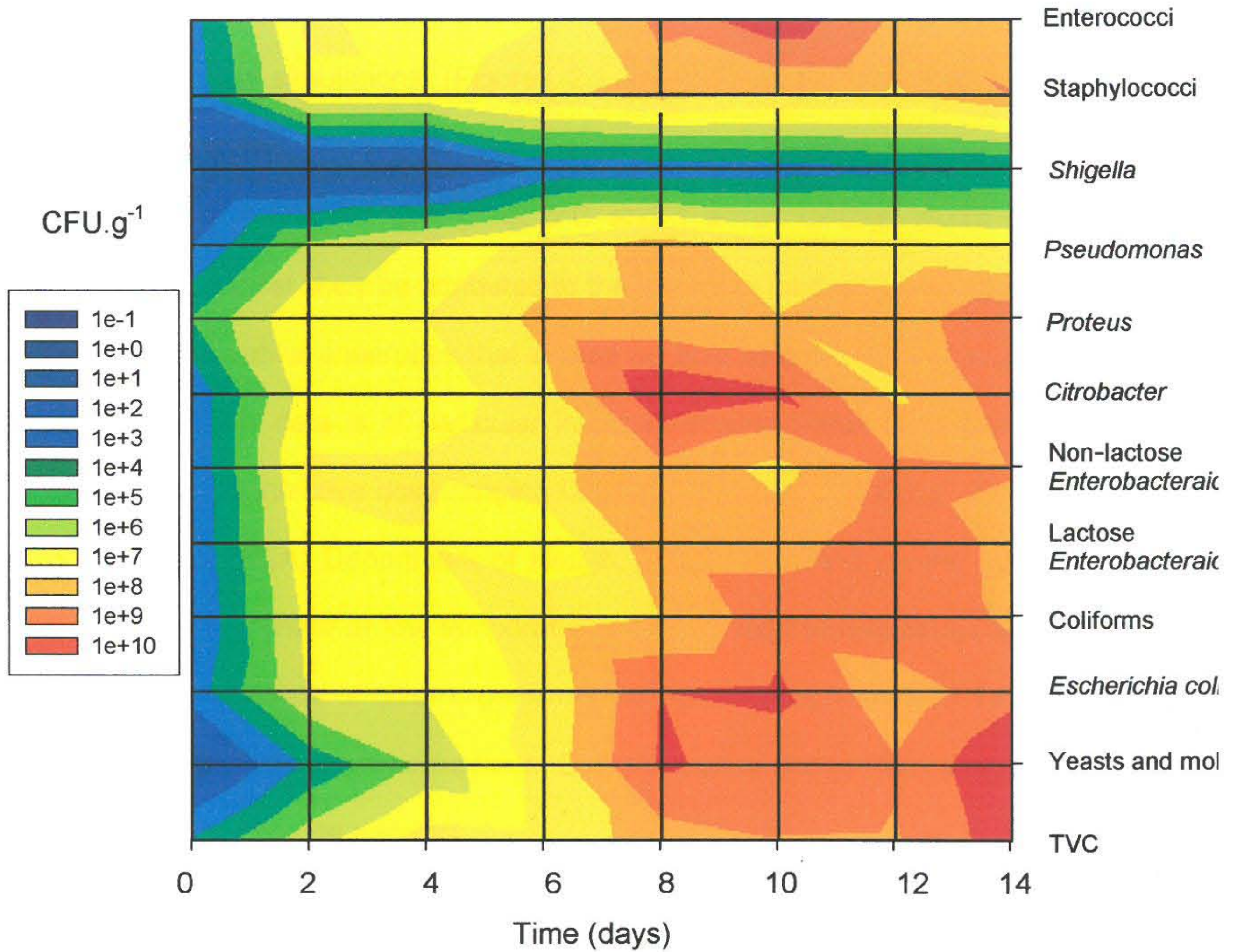

Figure 2.2 Growth patterns of different micro-organisms in vacuum-packed red meat stored at 18°C over a 14-day period

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storage, growth of organisms such as enterococci and *Proteus* sp. gradually declined after five weeks of storage. The pshychrofile, *Pseudomonas* did not grow as convincingly as was expected at the low temperatures.

In both storage simulations, (Figures 2.1 and 2.2) *Shigella* sp. showed limited growth during the first few days and weeks at both temperatures with average counts being 5.4 x 10^2 CFU.g^{-1} at 5ºC and 1 x 10^4 CFU.g^{-1} at 18ºC. These numbers can most likely be attributed to the low initial load of these organisms as they are facultative anaerobes that should be able to grow under the prevailing conditions. This data is of particular interest because these organisms have a relatively small infective dose ranging between 10^1 and 10^2 CFU.g^{-1} (Republic of South Africa (RSA) Department of Health, 2003). The TVC on the other hand were more prominent at low temperatures than at high temperatures compared to the predominance of other organisms. The lower growth rates of TVC at 5ºC probably resulted from lower residual O_2 consumption that in turn supported the survival of the obligate aerobes.

Compared to the other organisms, yeasts and molds also multiplied at slower rates at 5ºC than at 18ºC. At 18ºC the acceleration of mold and yeast growth was preceded by active bacterial growth that most likely resulted in a lower pH - an environment that favours fungal growth. Both pH and O_2 changes did not seem to influence the growth patterns of the lactose and non-lactose fermenting organisms. Likewise, *Citrobacter* sp. showed a similar pattern in both simulations

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although somewhat more pronounced at 18ºC. The faecal-associated bacteria (*Escherichia coli* and total coliforms) as well as *Staphylococcus* sp. also followed the same trend. *Escherichia coli* counts exceeded the national guideline of 1.0 x 10^1 CFU.g^{-1} (RSA, Department of Health, 2003) while *Salmonella* sp. were not detected.

2.4.2 Inter-relationships amongst various micro-organisms stored at 5ºC and 18ºC

In order to determine the exact relationships amongst various microbiota, Spearman's correlation coefficient was used to construct a correlation matrix using the following 12 variables: TVC, yeasts and molds, *Escherichia coli*, *Citrobacter*, *Proteus*, *P. aeruginosa*, lactose fermenting *Enterobacteriaceae,* non-lactose fermenting *Enterobacteriaceae*, coliforms, *Enterococcus*, *Staphylococcus* and *Shigella*. The purpose of this evaluation was to ascertain interdependency and possible synergy amongst the various genera during storage using inferential statistics.

At 5ºC, vacuum-packed red meat provided a growth environment that supported growth for a number of organisms. This became evident in the high (>0.90) correlations between the growth of fungi and TVC, *Pseudomonas* and aerobic plate counts, *Citrobacter* sp. and *Escherichia coli*, and *Staphylococcus* sp. with both *Shigella* sp. and total coliforms (Table 2.1). Though the growth of *Proteus*

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Table 2.1. Correlations amongst various … uum-packed storage

	TVC	YM	Ec	C	P	Pa	LfE	NlfE	Co	En	St	Sh
TVC		**0.97**	0.68	0.64	-0.48	**0.92**	0.25	**0.84**	0.72	**-0.78**	0.60	0.55
YM			**0.82**	**0.80**	-0.49	**0.85**	0.31	0.68	**0.85**	**-0.80**	0.75	0.71
Ec				**0.98**	-0.39	0.64	0.44	0.23	**0.78**	-0.69	0.71	0.71
C					-0.43	0.58	0.54	0.17	0.80	-0.72	0.74	0.75
P						-0.49	-0.51	-0.40	-0.35	**0.77**	-0.28	-0.26
Pa							0.43	**0.87**	0.45	**-0.81**	0.29	0.24
LfE								0.18	0.11	-0.69	0.03	0.03
NlfE									0.27	-0.63	0.12	0.05
Co										-0.55	**0.99**	**0.97**
En											-0.43	-0.40
St												**0.97**
Sh												

TVC (Total Viable Counts), YM (yeasts and molds), Ec (*Escherichia coli*), C (*Citroacter*), P (*Proteus*), Pa (*Pseudomonas aeruginosa*), LfE (lactose fermenting *Enterobacteriaceae*), NlfE (non-lactose fermenting *Enterobacteriaceae*), Co (coliforms), En (*Enterococcus*), St (*Staphylococcus*) and Sh (*Shigella*)

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sp. and enterococci showed a relatively strong positive correlation (r^2=0.77), both of these groups correlated negatively with the remaining organisms (r^2=-0.81 to -0.93). This interaction, viewed in the light of the growth patterns of these organisms, suggests possible competition for nutrients whilst another possibility could be the result of a negative reaction of these organisms to the changing environment.

The 18°C storage temperature showed interactions that differed vastly from those at the low temperature storage (Table 2.2). No strong negative correlations were observed while strong correlations (r^2 > 0.90) between the growth patterns of the following organisms were evident: *Staphylococcus* sp. with TVC, fungi with *Proteus* sp.; total coliforms with lactose fermenting *Enterobacteriaceae*; *Pseudomonas* sp. with *Citrobacter* sp.; *Proteus* sp. with TVC and fungi with TVC. These definite interactions suggest that little competition existed amongst the actively growing bacterial and fungal community, but rather a degree of synergy. Microbial successions due to the changing environment were not as obvious, as the majority of microbiota reached stationary phase close to the end of the sampling period.

In conclusion, the results of the study indicate that although proliferation of some microbes was limited at 5°C, and may be perceived as sensory intact, this may be misleading to consumers. High microbial loads at the onset of the study were indicative of poor handling practices of the product prior to vacuum-packing of

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Table 2.2 A summary of correlations amongst 18°C storage temperature

	TVC	YM	Ec	C	P	Pa	LfE	NlfE	Co	En	St	Sh
TVC		**0.96**	0.14	-0.10	**0.98**	-0.16	0.00	0.47	-0.10	-0.12	**0.98**	**0.85**
YM			0.32	0.16	**0.96**	0.11	-0.03	0.48	-0.14	-0.08	**0.99**	**0.83**
Ec				**0.78**	0.12	0.54	-0.13	0.00	0.08	0.76	0.25	0.28
C					-0.10	**0.95**	-0.12	0.03	-0.10	0.27	0.07	0.00
P						-0.14	0.03	0.50	-0.08	-0.15	**0.98**	**0.86**
Pa							-0.04	0.11	-0.14	-0.03	0.03	-0.06
LfE								**0.85**	**0.92**	-0.08	0.00	0.46
NlfE									0.68	-0.22	0.50	**0.78**
Co										0.29	-0.11	0.41
En											-0.11	0.08
St												**0.86**
Sh												

TVC (Total Viable Counts), YM (yeasts and molds), Ec (*Escherichia coli*), C (*Citroacter*), P (*Proteus*), Pa (*Pseudomonas aeruginosa*), LfE (lactose fermenting *Enterobacteriaceae*), NlfE (non-lactose fermenting *Enterobacteriaceae*), Co (coliforms), En (*Enterococcus*), St (*Staphylococcus*) and Sh (*Shigella*)

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the meat. Alternatively, although meat products are sensory entirely unacceptable after a period of time at elevated temperatures, they might not necessarily be prone to high numbers of pathogen species. To circumvent the presence of pathogenic microbes on fresh red meat and vacuum-packed red meat, Good Manufacturing Programmes (GMP) and Quality Control (QC) programmes should be properly implemented - a practice not in place at the abattoir under investigation. In addition, the use of atmospheric packaging is recommended in the studied abattoir to further minimise possible spoilage.

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CHAPTER 3

The distribution of *Staphylococcus* sp. on bovine meat from abattoir deboning rooms

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3.1 Abstract

In developing countries such as South Africa, *Staphylococcus aureus* has consistently been shown to be one of the most important micro-organisms responsible for food poisoning outbreaks. In this study the staphylococci in selected South African abattoirs were quantified, identified and further characterised in terms of coagulase types. The highest staphylococci counts (1.7 $X10^6$ $CFU.g^{-1}$) were observed in the meat from the high throughput (Grade A) abattoir during week 3. The counts exceeded the National Guidelines (10^2 $CFU.g^{-1}$) without exception and at least 50% surpassed the infective dose of 10^5 $CFU.g^{-1}$ determined for *S. aureus*. Species were dominated by *S. capatis, S. xylosus, S. auricularis, S. aureus* and *S. intermedius*. In terms of the coagulase types of *S. aureus,* type V was the most dominant and type VI the least. It became evident that the hygiene practices implemented by the abattoirs investigated in this study were not effective enough in reducing the contamination levels of the staphylococci from carcasses. It is therefore recommended that the sampled abattoirs revise their manufacturing strategies in order to reduce the levels of staphylococci contamination which have been shown to be transferred through food handlers, surfaces, equipment and the environment.

Keywords: *Staphylococcus;* coagulase typing; red meat; deboning

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3.2 Introduction

In recent years various staphylococci-contaminated food products that include red meat have been implicated in food poisoning outbreaks (Uche and Agbo 1985; Benito *et al.*, 2000; Atanassova *et al.*, 2001; Hein *et al.*, 2001; Miwa *et al.*, 2001; Lior *et al.*, 2003). Because of the ubiquitous nature of organisms belonging to the *Staphylococcus* genus, contamination of food products in processing plants has often been shown to result from food handlers and asymptomatic carriers suffering from infected skin lesions (Desmarchelier *et al.*, 1999; Mosupye and Von Holy, 2000; Hein *et al.*, 2001; Nagase *et al.*, 2002; Palomares *et al.*, 2003; Nel *et al.*, 2004).

The *Staphylococcus* genus consists of 38 species of which *Staphylococcus aureus* is the most notorious (Edwards *et al.*, 2001; Nel *et al.*, 2004). This species is often referred to as it is used as an indicator of personal hygiene and is known to produce harmful enterotoxins (Edwards *et al.*, 2001). With regard to the presence of *S. aureus* in red meat, South African legislation stipulates a maximum of 10^2 CFU.g^{-1}, whilst 10^5 -10^8 CFU.g^{-1} of this organism per gram of meat is sufficient to produce toxins (Agricultural Research Council (ARC) 1998; Meat Inspectors Manual, 2000; Hein *et al.*, 2001; South African Meat Industry Companies (SAMIC) 2002). These toxins are thermo-stable although the vegetative cells of *S. aureus* can readily be destroyed by high temperatures. It is known that once *S. aureus* is introduced into the food processing plant, this

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organism can persist in biofilms for long periods of time and can be transferred from one point to another causing cross-contamination (Hein *et al.*, 2001; Hecker *et al.*, 2002).

South African abattoirs are graded according to various throughput rates, with the grade A abattoirs having the highest throughput of more than one hundred slaughtering units per day. Grade E abattoirs, on the other hand, have the lowest throughput of one to eight slaughtering units per day, while the throughput of the remaining grades (B, C and D) falls between the throughput ranges of grade A and E abattoirs (Van Zyl, 1998; SAMIC, 2000). These abattoirs process the 12.4 kg of red meat that is consumed per person per annum in South Africa. In the majority of these plants, the carcasses are extensively handled during the deboning process in designated deboning rooms.

In addition to poorly sanitized conveyer belt surfaces and the carcass being contaminated with staphylococcal species originating from the animal itself, the increased contact of the handlers with the carcasses during this process has proven to be a major route of contamination (ARC, 1998; Midelet and Carpentier, 2002; Nel *et al.*, 2004). Nel *et al.* (2004) suggest the deboning process of a high throughput abattoir in South Africa as the major processing step that contributes to significant amounts of staphylococci. The latter study further indicates that *S. aureus* counts often exceed the legislative limits (Nel *et al.*, 2004).

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In the present study the staphylococci associated with red meat originating from the various grades of abattoirs used in South Africa were enumerated and identified. The study further characterised the isolated *Staphylococcus aureus* based on its coagulase typing properties. The purpose of the study is to cast light on the level of contamination in the various abattoirs in order to ascertain the role that throughput and infrastructure play in the contamination process. By focusing on staphylococci, specific emphasis is placed on the contamination by food handler's.

3.3 Materials and methods

3.3.1 Sampling site and procedure

Samples of deboned bovine meat were obtained from four differently graded (A, B, C and D) South African red meat abattoirs in the Free State Province (van Zyl, 1998). Each plant was visited on a weekly basis for five consecutive weeks during late spring. During each sampling day, samples were aseptically collected every hour on a random basis for a period of five hours from red meat processed in the deboning room prior to vacuum packaging. The collected samples were transported at 0°C to the laboratory without delay.

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3.3.2 Sample treatment

Ten-gram portions of the samples were aseptically homogenised in a Stomacher (Seward 400) for 2 minutes (Nortjé *et al.*, 1999), in 90 ml sterile peptone water (Biolab, Midrand, SA). Consecutive serial dilutions were prepared and 0.1ml aliquots plated onto media using the spread-plate technique (Herbert, 1990). For enumeration of Total Viable Counts (TVC), Plate Count Agar (PCA) plates were incubated at 25ºC for 72 hours (Vanderzant and Splittstoesser, 1992) while Baird-Parker Agar (BPA) plates were incubated at 35ºC for 48 hours for enumeration of *Staphylococcus* sp. (Nikanen and Aalto, 1978). All *S. aureus* colonies were confirmed using the Rapid Latex Agglutination Test (Slidex Staph Plus test kit, Bio Mérieux, France; Personne *et al.*, 1997; van Griethuysen *et al.*, 2001).

3.3.3 *Staphylococcus* species identification

Colonies from Baird-Parker Agar plates which were not *S. aureus* were plated on Blood Agar and incubated for 24 hours at 35ºC. These *Staphylococcus* sp. were identified using the API-Staph system (Nagase *et al.*, 2002) and APILAB software in accordance to the manufacturer's procedure (Bio Mérieux, France).

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3.3.4 Coagulase typing of *Staphylococcus aureus*

Some authors have suggested the division of *S. aureus* into coagulase types by identifying the origin of each coagulase type and listing their lethality in terms of such types (Shimizu *et al.*, 2000; Nagase *et al.*, 2002). The method of classifying *S. aureus* according to its coagulase types was consequently used to aid epidemiological investigations into staphylococcal food poisoning (Shimizu *et al.*, 2000; Nagase *et al.*, 2002). In this study, coagulase typing of *S. aureus* isolates was carried out as prescribed by Ushioda *et al.*, (1981) using a coagulase typing kit (Denka Seiken, Tokyo). Neutral rabbit antisera specific to eight coagulase types, I to VIII, were used (Ushioda *et al.*, 1981; Shimizu *et al.*, 2000; Nagase *et al.*, 2002).

A positive control of *S. aureus* and negative control of *S. epidermitis* were included. Aliquots (0.1ml) of each antiserum and normal rabbit serum (control) were added to 0.1ml of the supernatant obtained from an 18-hour culture of each test isolate, incubated at 35ºC for 1 hour and added to 0.2ml aliquots of rabbit plasma. Inhibition of coagulase after further incubation at 35ºC for at least 1 hour indicated the coagulase type.

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3.4 Results and discussion

3.4.1 Meat-borne Total Viable Counts (TVC) and Staphylococci

The quantity and distribution of TVC and *Staphylococcus* sp. are shown in Figure 3.1. For abattoir A, weeks 1, 2 and 5 showed similar numbers of TVC while slightly higher counts were observed during week 3 and the highest in week 4 (1.3×10^9 $CFU.g^{-1}$). In all cases staphylococci counts were lower than TVC except during week 5 when the majority of TVC were dominated by staphylococci (Figure 3.1, A). In abattoir B the average TVC for all five weeks was 2.7×10^8 $CFU.g^{-1}$ and for staphylococci 5.5×10^5 $CFU.g^{-1}$ (Figure 3.1, B). These were relatively similar to the average counts found in abattoir A (Figure 3.1, A). Abattoir C (Figure 3.1, C) however, showed both TVC and staphylococci to be lower (7.3×10^4 and 3.6×10^3 $CFU.g^{-1}$ respectively) compared to abattoirs A and B, although the highest TVC numbers in abattoir C were 2.7×10^6 $CFU.g^{-1}$ during week 2. Abattoir D (Figure 3.1, D) was similar to abattoirs A and B in terms of both TVC and staphylococci.

In general, all the abattoirs exceeded the TVC limit of 10^5 $CFU.g^{-1}$ for red meat as suggested by Jay (2000). Furthermore, levels of staphylococci from all abattoirs exceeded the National Guideline of 10^2 $CFU.g^{-1}$ for *S. aureus* in fresh red meat (ARC, 1998; Meat Inspectors Manual, 2000, Department of Health, 2003). The results were compared to the study of Nel *et al.* (2004) on bacterial populations

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associated with red meat in a high throughput (Grade A) beef abattoir where staphylococci counts ranged from 3.8 x 10^3 to 2.42 x 10^5 CFU.g^{-1}. These counts found by Nel *et al.* (2004) were lower compared to the present study where staphylococci counts averaged 1.25 x 10^5 CFU.g^{-1} in the high throughput (Grade A) abattoir. Apart from these results, 60% of the samples from this abattoir exceeded the 10^5 CFU.g^{-1} staphylococcal infective dose, compared to the study by Nel *et al.* (2004) where the authors found that 50% of samples exceeded the infective dose (ARC, 1998; Hein *et al.*, 2001).

The data obtained in this study furthermore exceed the levels recorded from various abattoirs and retailers during another surveillance study undertaken on the safety and hygiene of meat in the North West Province of South Africa. In the latter study graded abattoirs A, B, C, D and E showed average counts of log 2.25 CFU.g^{-1} (ARC, 1998). According to Forsythe (2000), such high counts suggest a possible food poisoning hazard because certain *Staphylococcus* sp. produce toxins when exposed to storing and cooking and as a result, illness may occur after consumption of such food (Nel *et al.*, 2004).

3.4.2 Species identification of staphylococci

It is known that staphylococci of human and animal origin differ with regard to their predominance and characteristics (Nagase *et al.*, 2002), and studies into the sources of contamination by *Staphylococcus* species are limited (Devriese *et al.*,

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Table 3.1 Staphylococcal species on red meat from various abattoirs

Abattoirs	Abattoir A		Abattoir B		Abattoir C		Abattoir D		
Species	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**Coagulase type**
S. aureus	10	24.4	19	28.8	7	30.5	3	15.8	+
S. auricularis	1	2.4	11	16.7	7	30.5			-
S. capitis	14	34.1	9	13.6	1	4.3	2	10.5	-
S. chromogens			4	6.1	1	4.3			-
S. cohnii cohnii			3	4.5					-
S. epidermidis	1	2.4							-
S. haemolyticus					1	4.3			-
S. homonis			1	1.5					-
S. intermedius	3	7.4	6	9.1	2	8.8	1	5.3	+
S. lentus			3	4.5			3	15.8	-
S. saprophyticus	2	4.9	4	6.1					-
S. sciuri					1	4.3	4	21.1	-
S. warneri					3	13			-
S. xylosus	10	24.4	6	9.1			6	31.6	-
Total	41	100	66	100	23	100	19	100	150

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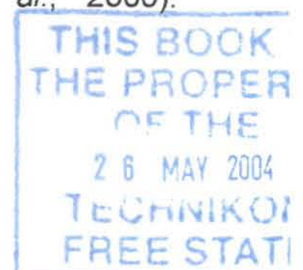
1985). Table 3.1 shows the *Staphylococcus* species isolated on red meat from high and low throughput abattoirs. *Staphylococcus capitis,* which is found on the skin, hair and in the nostrils of warm blooded animals including humans (Euzeby, 2003; Lior *et al.*, 2003) was found to be dominant in abattoir A (34.1%). *Staphylococcus epidermidis,* which is normally found on the forehead, axillae, arms and legs of humans was found to be present only in abattoir A (2.4%). It has been reported that 63.8% of human *Staphylococcus* carriers carry *S. epidermidis* in and on their bodies (Nagase *et al.*, 2001). The species *S. haemolyticus* and *S. warneri,* reported to be from the same origin as *S. epidermidis* (Glendening, 2002), were isolated from abattoir C only (Table 3.1). *Staphylococcus auricularis,* which is associated with the human ear (Euzeby, 2003), was found to be present in abattoirs A, B, and C (2.4%, 16.7% and 30.5% respectively).

Table 3.1 furthermore shows that the species *Staphylococcus sciuri* occurred only in abattoirs D and C (4.3% and 21.1% respectively), while *S. xylosus* was present in all abattoirs except C. These two species are known to be associated with the teat skin of various domestic and wild animals (Nagase *et al.*, 2000). *Staphylococcus chromogens,* which is predominantly found in sheep (Hogg and Lehane, 1999), was present only in abattoirs B and C (6.1% and 4.3% respectively), as was the case with *S. cohnii. Staphylococcus lentus* occurred in abattoirs B and D (4.5% and 15.8% respectively). These two species are primarily associated with the skin of chickens (Nagase *et al.*, 2000).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Staphylococcus saprophyticus, isolated from abattoirs A and B (4.9% and 6.1% respectively), has been reported as the most often encountered causative agent of urinary tract infections and has been isolated particularly from young, sexually active females (Martineau *et al.*, 2000). The presence of this organism may point to shortcomings in the facility design (e.g. toilet facilities) as well as to a lack of training in proper personal hygiene (Nel *et al.*, 2004). *Staphylococcus saprophyticus* has however, also previously been isolated from bovine nostrils (Euzeby, 2003), indicating possible inadequacies in the slaughtering process. Another species, *S. intermedius,* is known to originate from animals and less frequently from humans; it is also known to be associated with dogs (Nagase *et al.*, 2000; Euzeby, 2003). This species was isolated in all the abattoirs A, B, C and D (7.4%, 9.1%, 8.8% and 5.3% respectively).

The presence of *S. homonis* (1.5%, abattoir B) is commonly associated with human blood due to contamination of wounds by bot fly (*Dermatobia homonis)* (Sancho *et al.*, 1996; Euzeby, 2003). This raised the question as to whether workers were sometimes working with open wounds and failing to report cuts, injuries or illnesses to management. From abattoir A 75% isolates, abattoir B 60% isolates, abattoir C 91% isolates and abattoir D 31% isolates were of human origin, thus suggesting that human handling leads to the spreading of the majority of species throughout the final product.

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3.4.3 *Staphylococcus aureus* and its coagulase types

Coagulase types of *S. aureus* have previously been used to investigate the sources of the organism in various environments. Of the eight coagulase types, coagulase types III and VI have been reported to be of bovine origin whilst types II and VIII are non-host specific (Kakinohana *et al*, 2002; Nagase *et al.*, 2002). Coagulase type IV originates from poultry ecovars and type VII has been isolated from food although its origin, as well as that of coagulase types V and I, is still unclear (Shimuzi *et al.*, 2000; Kakinohana *et al*, 2002; Nagase *et al.*, 2002). Of all these coagulase types, only types I and VIII produce toxins. The remaining types are non-toxin producers (Shimuzi *et al.*, 2000).

In the present study *Staphylococcus aureus* isolates were found to be distributed throughout the four abattoirs in percentages of 24.4, 28.8, 30.5 and 15.8 (abattoirs B, A, C, and D respectively) as shown in Table 3.1. The mean counts for *S. aureus* in abattoirs A, B, C and D were 1.25×10^5, 1.70×10^4, 1.11×10^3 and 5.25×10^4 $CFU.g^{-1}$ respectively. Coagulase type I was evenly distributed throughout abattoirs A, B and C whereas coagulase type II was isolated only from abattoirs B and C (21.1% and 14.3% respectively). Table 3.2 furthermore shows that type III was primarily isolated from abattoir A (30%) followed by a relatively even distribution amongst the remaining abattoirs. Abattoir B was dominated by coagulase type IV (26.4%) whilst the remaining isolates of this type were found only in abattoir A. Type V was prevalent in abattoirs C (42.8%) and B

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Table 3.2 *Staphylococcus aureus* coagulase types isolated on red meat from various abattoirs

Abattoirs	Abattoir A		Abattoir B		Abattoir C		Abattoir D	
Type(s)	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
I	1	10	1	5.4			1	33.4
II			4	21.1	1	14.3		
III	3	30	1	5.4	1	14.3	1	33.3
IV	1	10	5	26.4				
V	1	10	2	10.5	3	42.8	1	33.3
VI	1	10	1	5.4				
VII	1	10	2	10.5	1	14.3		
VIII	2	20	3	15.8	1	14.3		
Total	10	100	19	100	7	100	3	100

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(10.5%) and evenly distributed amongst the remaining abattoirs. Type VII was absent in abattoir D but found in the remaining abattoirs in the order of 10%, 10.5%, and 14.3% for abattoirs A, B and C. Coagulase type VIII predominated in abattoir B (15.8%) with 20% of the isolates from abattoir A and 14.3% from abattoir C (Table 3.2).

In comparison with other studies, Shimizu *et al.* (2000) report that type VII accounted for 70% of the existing coagulase types, followed by type III with 12 %, type VI with 3% and type II, 1%. In another study Nagase *et al.* (2002) report that coagulase type VI was the most predominant, accounting for 77% of the total strains studied. In the present study, however, type V predominated followed by types III, IV and VIII. The remaining coagulase types were isolated in low numbers. It was thus evident that the coagulase types differed, based on geographical location and environmental conditions. Encouraging was the fact that toxin-producing coagulase types (I and VIII) were isolated in low numbers only.

In general, abattoir C carcasses were the least contaminated compared to the other abattoirs investigated in this study as a result of TVC and staphylococci counts which were low compared to the rest of the abattoirs. The results further showed that contamination of the carcasses by staphylococci is caused by a variety of sources such as the food handlers themselves, the surfaces and utensils used during slaughtering as well as from the animal during slaughtering.

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Species such as *S. auricularis*, *S. aureus*, *S. epidermidis*, *S. capatis*, *S. warneri*, and *S. haemolyticus* were most likely transmitted to the meat via the food handlers because of a lack of proper personal and process hygiene. Sources of *S. saprophyticus* may be linked to inadequate toilet facilities. The results further suggested that species of animal origin such as *S. lentus*, *S. conhii conhii*, *S. chromogens*, *S. xylosus*, and *S. sciuri* were possibly transferred from domestic animals to food handlers and then back to carcasses, or alternatively that they originated from the animals during slaughtering.

Finally, it emerged that throughput rate has no obvious influence on the contamination levels of the product by staphylococci as no definite relationship could be observed between the numbers, species or types of these organisms amongst the various low and high throughput abattoirs. Management commitment, infrastructure and training of the food handlers appear to be the primary issues to be addressed in order to curb undesirable contamination of the product.

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CHAPTER 4

The distribution of staphylococci in bioaerosols from red meat abattoirs

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4.1 Abstract

High bioaerosol concentrations consisting of spoilage microbiota in the processing environment have been reported to reduce the quality and shelf-life of perishable foodstuffs. A lack of documented literature on the distribution of such bioaerosols has however, led to the underestimation of their impact. In this study the deboning rooms of selected South African red meat abattoirs were investigated for airborne concentrations of staphylococci, their species distribution as well as coagulase types of *Staphylococcus aureus* in particular. Mean staphylococcal bioaerosol concentrations varied considerably amongst the abattoirs investigated, ranging from 8-3.5 x 10^3 CFU.m^{-3}. *Staphylococcus xylosus* and *S. saprophyticus* were found to be the most abundant species in the air of the deboning rooms. *Staphylococcus aureus* coagulase types III and VIII were furthermore found to predominate the isolated coagulase types. Based on the ecology of the bacterial groups, the authors propose probable sources of staphylococcal bioaerosols and suggest appropriate strategies to be developed in red meat abattoirs in order to reduce the levels of airborne-related pathogens.

Keywords: Staphylococci, bioaerosols, deboning room, abattoir.

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4.2 Introduction

Contamination of foodstuffs via the air is generally caused by viable airborne contaminants originating from biological sources (bioaerosols) (Lutgring *et al.*, 1997; Whyte *et al.*, 2001; Beggs, 2002). Bioaerosols can be liquid, solid, carried on another particle or suspended in a liquid droplet, and may comprise bacterial spores, cells, fungi or fungal spores, antigens, viruses, plant pollens, toxins and/or faecal material (Radmore, 1986; Lutgring *et al.*, 1997; Cundith *et al.*, 2002). When all these substances are distributed in the air, they can serve as a feasible route for food contamination and can ultimately affect the health of both the food handlers and consumers (Lutgring *et al.*, 1997). Information on the bioaerosol composition of food processing plants is however very limited, mainly because laboratories lack proper equipment and expertise to perform bioaerosol surveys. As a result knowledge of the contribution of the airborne microbiota to the contamination of food products remains limited.

A number of authors have recognised the ventilation system as a possible reservoir that distributes bioaerosols in meat processing plants (Whyte *et al.*, 2001; Cundith *et al.*, 2002). In addition, employees may be distributors of contaminants through their clothes, skin, hair, respiratory tract (coughing and sneezing), and faecal matter (Lutgring *et al.*, 1997; Chambers, 2001; Cundith *et al.*, 2002). According to Beggs (2002), food handlers are the primary sources of indoor bioaerosols in the food industry. Other sources that are indirectly related to the bioaerosols are contaminants from waste handling and disposal, fungal or

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bacterial growth in the building, maintenance problems and poor sanitation practices. In addition, seasonal and weather-related factors such as the geographical location are known to influence the bioaerosols within the abattoir (Ren and Frank, 1992; Lutgring *et al.*, 1997; Pastuszka *et al.*, 2000; Chang *et al.*, 2001; Cundith *et al.*, 2002). According to Zadoks *et al.,* (2002), airborne micro-organisms may be of human origin from purulent discharges of an infected finger, eye, abscesses, facial eruptions, nasopharyngeal secretions or normal skin.

The most prominent pathogenic bacteria found to predominate in indoor bioaerosols are members of the *Staphylococcus* genus (Wieser and Busse, 2000). This genus occurs naturally on the skin, as well as on skin glands and mucous membranes of warm-blooded animals (Wieser and Busse, 2000; Nagase, 2002a; Nagase et al., 2002b). *Staphylococcus aureus* constitute about 10% of the nasal cavity bacteria of healthy humans and occur at levels of 1×10^{-1}-1×10^{-2} $CFU.m^{-3}$ in the environment (Sheretz *et al.*, 2001). Because of their ubiquitous occurrence in nature, staphylococci have been isolated from fresh water, meat, milk, cheese, soil, seawater, dust and the air (Wieser and Busse, 2000; Nel *et al.*, 2004). Although extensive literature exists on the quality and safety of meat, milk, cheese and water, little information exists on the microbiological aspects of indoor air associated with red meat abattoirs (Pastuszka *et al.*, 2000).

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The majority of the larger red meat abattoirs boast deboning rooms for the removal of retail cuts from the carcasses. During this stage the product undergoes extensive handling and exposure to surfaces and utensils (Nel *et al*, 2004). As a result, deboning rooms are prone to several factors that can contribute to the distribution of airborne staphylococci. These include 1) conveyer systems and cutting tables; 2) movement and actions of food handlers especially through breathing, speaking, sneezing, or coughing; 3) water drains and splashing caused during high-pressure water washing of the floor; and 4) the ventilation system (Ren and Frank, 1992).

The aim of this study is to quantify the airborne staphylococci in the deboning rooms of selected South African red meat abattoirs of various sizes and throughput rates. The staphylococci species found in the air will be further characterised and *Staphylococcus aureus* coagulase types assessed in order to shed light on the airborne distribution of these micro-organisms. Suggestions will finally be made as to the role of the air environment as vector of staphylococcal contamination.

4.3 Materials and methods

4.3.1 Sampling site

Bioaerosol samples were collected from four differently graded (A, B, C and D) South African red meat abattoirs in the Free State Province. Grade E abattoirs

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were excluded as they do not usually contain deboning areas (Van Zyl, 1998). Each plant was visited on a weekly basis for five consecutive weeks.

4.3.2 Sampling procedure

During each sampling interval, samples were collected aseptically from various locations in the deboning room, every hour for a period of five hours. Samples were collected using a single stage microbial air sampler (SAS Super 90; Clark *et al.*, 1983; Donham *et al.*, 1986; Haglind and Rylander, 1987; Donham *et al.*, 1989; Cormier, 1990; Heedrich, 1991; Thorne *et al.*, 1992). The air sampler was pre-calibrated at 200 $dm^3.min^{-1}$ and all removable components were pre-autoclaved and disinfected with 70% ethanol between sampling runs. The air sampler operates by directly collecting airborne microbes onto 55mm RODAC plates through impaction (Theron, 2003). The collected samples were stored at 0ºC during transportation to the laboratory.

4.3.3 Sample treatment

For enumeration of Total Viable Counts (TVC), Plate Count Agar (PCA) plates were incubated at 25ºC for 72 hours (Vanderzant and Spittstoesser, 1992) whilst Baird-Parker Agar (BPA) plates were incubated at 35ºC for 48 hours for enumeration of *Staphylococcus* sp. (Nikanen and Aalto, 1978). Typical *S. aureus* colonies were confirmed using the Rapid Latex Agglutination Test (Slidex Staph

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Plus test kit, Bio Mérieux, SA; Griethuysen *et al.*, 2001). All the analyses were done at least in duplicate.

4.3.4 Staphylococcal species identification

Staphylococcus sp. which were not *S. aureus* were plated on Blood Agar for 18-24 hours and incubated at 35ºC. These *Staphylococcus* species were identified using the API-Staph system (Nagase, 2002a; Nagase *et al.*, 2002b) and APILAB software in accordance to the manufacturer's procedure (Bio Mérieux, SA).

4.3.4 *Staphylococcus aureus* coagulase typing

Coagulase typing was carried out according to the manufacturer's procedure (Ushioda *et al.*, 1981) using a Coagulase Typing Kit (Denka Seiken, Japan). Neutral rabbit antisera specific to eight coagulase types (I to VIII) were used (Ushioda *et al.*, 1981; Shimizu *et al.*, 2000). Negative controls of *S. epidermitis* and positive controls of *S. aureus* were used. Aliquots (0.1ml) of antisera as well as normal rabbit serum (as a control) were added to 0.1ml of supernatant obtained from a 12-hour culture of each test isolate and incubated at 35ºC for 1hour. Aliquots (0.2ml) of rabbit plasma were added and inhibition of coagulase after further incubation at 35ºC for at least 1hour indicated the coagulase type. Strains of which the coagulase activity was not neutralized by the set of antisera,

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and strains that reacted to more than two specific sera were designated non-typable (Shimizu *et al.*, 2000).

4.4 Results and Discussion

4.4.1 Distribution and patterns of microbiota

The distribution of the airborne Total Viable Counts (TVC) in the deboning rooms of various red meat abattoirs is shown in Figure 4.1. Apart from isolated TVC counts that were notably high in abattoir B during the second hour of week 1 (1.2×10^4 CFU.m^{-3}), the counts in the remaining abattoirs were relatively similar. The lowest counts (1.5×10^1 CFU.m^{-3}) during this week were noted in abattoir A during the fourth hour of sampling. During week 2, TVC in abattoir B remained high compared to the other abattoirs, with the second hour still showing the highest counts (3.6×10^3 CFU.m^{-3}). However, the counts decreased during the remaining hours at the same abattoir. Figure 4.1 further shows that during week 3, TVC were comparatively low in abattoir A with the lowest counts of 2.5×10^1 CFU.m^{-3} noted during the second hour, compared to the other abattoirs which showed higher counts at this interval. During this week, the counts observed in abattoir D increased steadily from the first hour, reaching the highest counts during the fifth hour of sampling.

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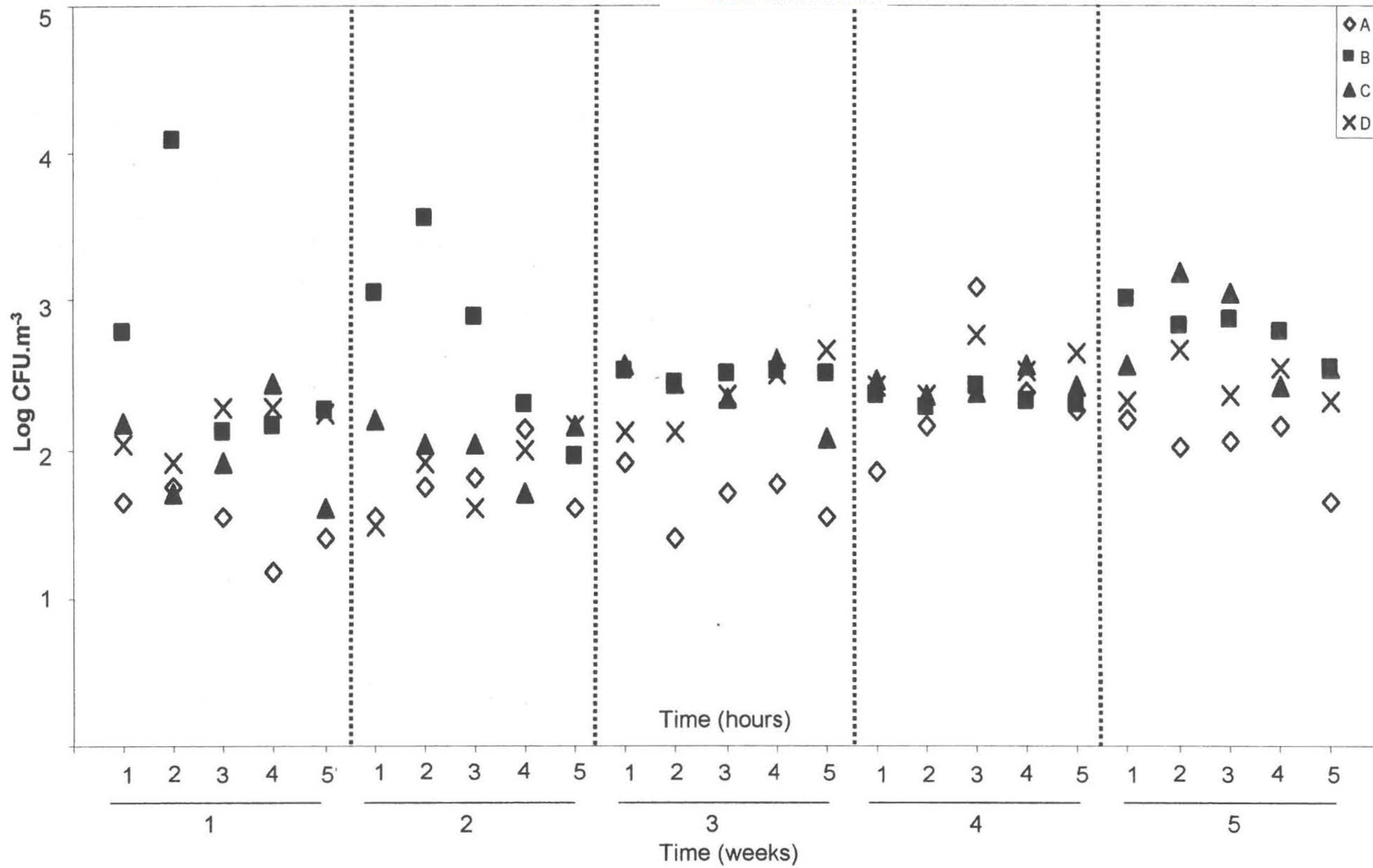

Figure 4.1 The patterns and distribution of Total Viable Counts (TVC) during various sampling intervals of hours and weeks. Abattoir A (A), Abattoir B (B), Abattoir C (C) and Abattoir D (D)

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Total Viable Counts (TVC) during week 4 were relatively similar, although the lowest counts (7×10^1 CFU.m^{-3}) were noted during the first hour in abattoir A. The highest counts (1.2×10^3 CFU.m^{-3}) were observed at the same abattoir during the third hour of sampling. The last week (week 5), showed a slightly different pattern, with abattoir C showing high counts (1.5×10^3 and 1.3×10^3 CFU.m^{-3}) during the second and third hours respectively. During the same week, abattoir A showed lower counts throughout the sampling intervals with the lowest counts (4.5×10^1 CFU.m^{-3}) observed during the last hour. In general, the airborne TVC counts appeared relatively similar throughout the sampling intervals of months and days with only a few exceptions which were distributed between the values of 10^1 and 10^3 CFU.m^{-3}. Apart from minor fluctuations amongst the sampling intervals of hours and weeks, no definite pattern could be observed that could suggest a time during the day, or over a month, when notable increases or decreases occurred in the TVC bioaerosol composition that could be related to a particular processing activity.

The distribution and patterns of the airborne staphylococci counts in the deboning rooms of various red meat abattoirs are depicted in Figure 4.2. Compared to the TVC bioaerosols (Figure 4.1) the staphylococci showed notably more fluctuations amongst both abattoirs and sampling intervals although definite patterns related to sampling intervals could similarly not be observed. The staphylococci counts in abattoir C remained relatively constant throughout the sampling intervals of week 1 (Figure 4.2). The highest counts (6.0×10^4 CFU.m^{-3}) were recorded during

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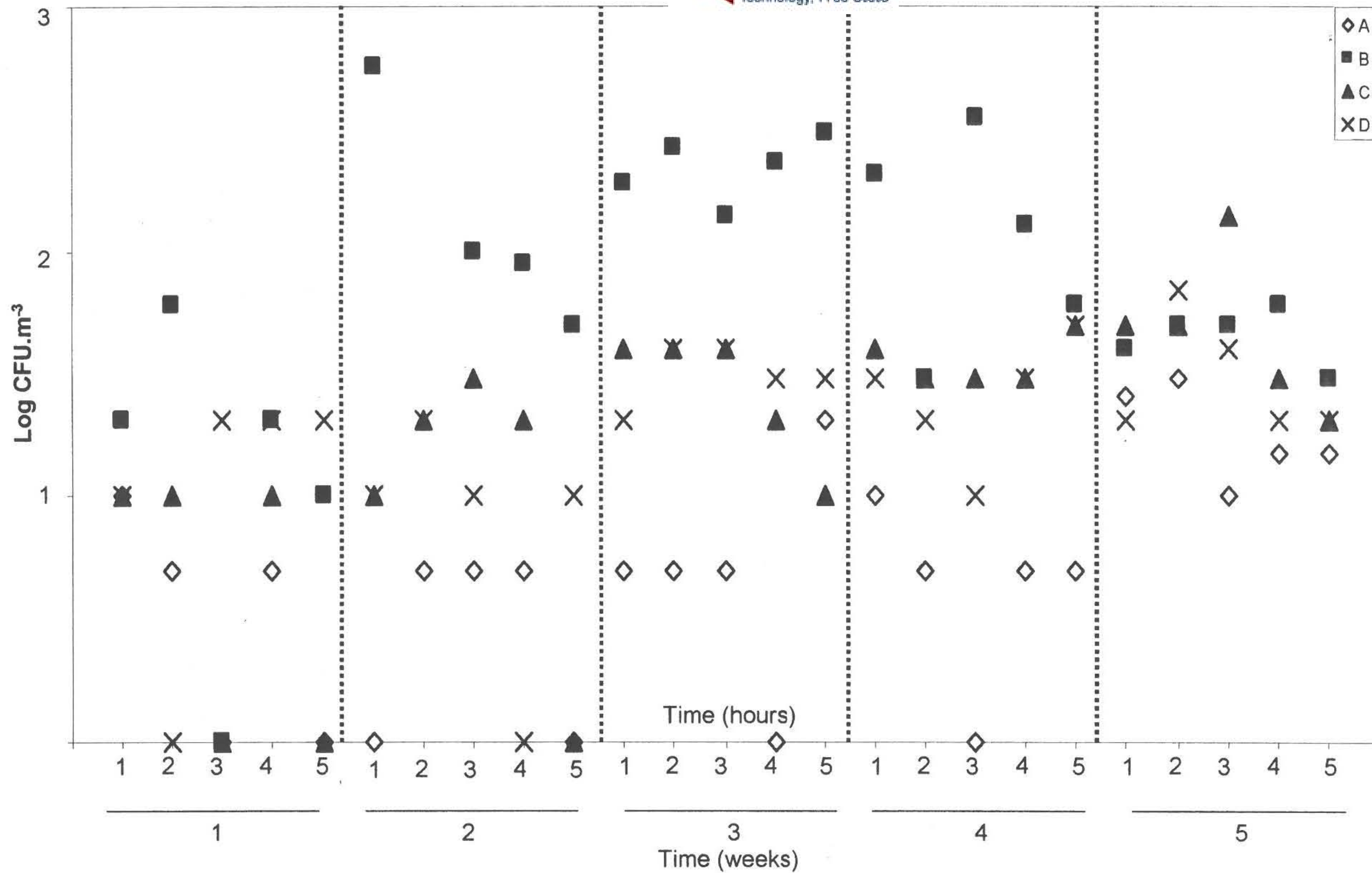

Figure 4.2 The patterns and distribution of staphylococci during various sampling intervals of hours and weeks. Abattoir A (A), Abattoir B (B), Abattoir C (C) and Abattoir D (D)

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week 2 in abattoir B during the second hour. The second week showed relatively high counts (1.02×10^{3} CFU.m^{-3}) in abattoir B during the second hour although the remaining counts decreased afterwards. The remaining abattoirs showed a similar pattern, except for abattoir D which showed an uneven distribution of counts throughout the sampling hours of this week (Figure 4.2).

During weeks 3 and 4 the bioaerosol counts of abattoir B predominated with the lowest counts observed in abattoir A. Abattoirs C and D showed a relatively similar pattern to abattoir C (Figure 4.2). The highest (3.50×10^{2} CFU.m^{-3}) and lowest (1 CFU.m^{-3}) counts were recorded during the third hour for abattoirs A and B respectively during week 4. During the last week the counts were relatively similar in all the abattoirs.

To shed light on the overall performance of the abattoirs the mean distribution of Total Viable and staphylococcal counts amongst the abattoirs are depicted in Figure 4.3. The results show that the counts in abattoir A were the lowest (1.3×10^{2} and 8×10^{0} CFU.m^{-3} respectively) for both TVC and staphylococci. Average values for TVC and staphylococci were 1×10^{3} and 1.6×10^{2} CFU.m^{-3} respectively for abattoir B, which were the highest values recorded of all four abattoirs. Abattoir C showed respective average counts of 3×10^{2} and 3×10^{1} CFU.m^{-3} for TVC and staphylococci whereas abattoir D showed respective mean counts of 3.1×10^{2} and 2.4×10^{1} CFU.m^{-3} for TVC and staphylococci.

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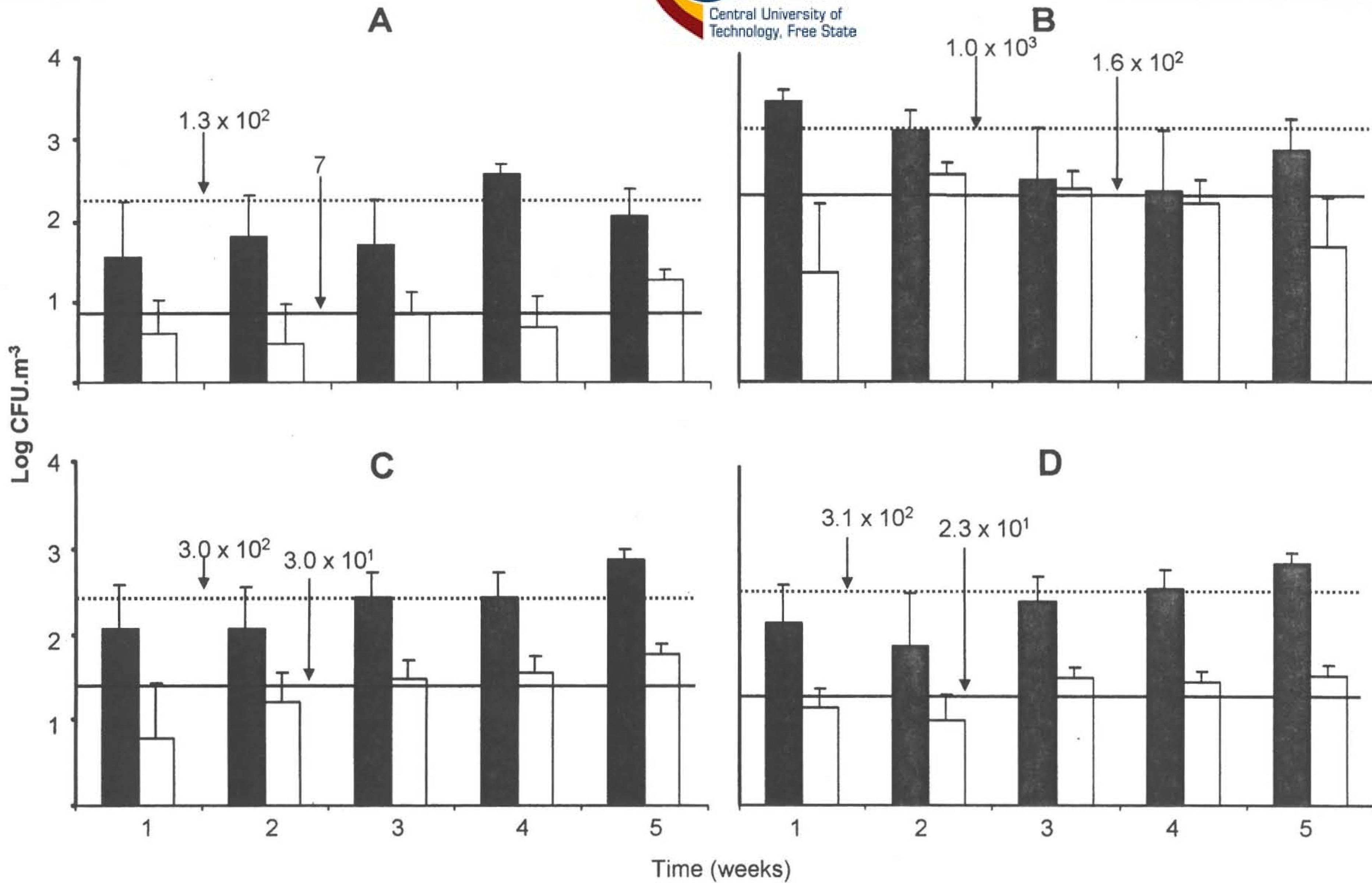

Figure 4.3 Average Total Viable Counts (TVC) and *Staphylococcus* counts from various red meat abattoirs. TVC (■) and Staphylococcus (□). Average TVC (········); *Staphylococcus* (——)

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The counts found in the various abattoirs did not exceed the guideline which was suggested by Jensen and Shafer (1998) for ubiquitous bacterial aerosols. However, the relatively low numbers recorded in this study do not necessarily indicate a clean and healthy environment as counts of less than 1 x 10^2 CFU.m^{-3} have been shown to be unhealthy for immuno-suppressed individuals (Jensen and Schafer, 1998).

4.4.2 Species of staphylococci isolated from bioaerosols

The airborne *Staphylococcus* species in the deboning rooms of the various abattoirs are shown in Table 4.1. The species *Staphylococcus capatis* generally found on the skin of human (Euzeby, 2003) was isolated in abattoirs A (2.5%), B (23%) and C (35.3%) respectively. *Staphylococcus caprae* which, amongst others, originates from the milk of mastitis-free goats (Alligent *et al.*, 2000), was isolated only in abattoir D. A third species, *Staphylococcus chromogens,* which is dominant in high concentrations in sheep (Hogg and Lehane, 1999) was also found only in abattoir D (Table 4.1). *Staphylococcus conhii* was isolated in all the abattoirs although predominantly in abattoir C (35.5%). This organism has been known to occur in abundance on the skin of chickens (Nagase *et al.*, 2002a; Nagase *et al.*, 2002b). *Staphylococcus epidermidis,* found only in abattoir D, has been shown to originate from the forehead, axillae, arms and legs of human. In addition, *S. epidermidis* was reported to be present in the bodies of 63.8% of *Staphylococcus* carriers in the USA and is the second most common airborne

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Table 4.1 Staphylococcal species found in the air of deboning areas of various abattoirs

Abattoirs	**Abattoir A**		**Abattoir B**		**Abattoir C**		**Abattoir D**		
Species	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**Coagulase typ**
S. aureus	2	5.9	4	13			5	23.8	+
S. capitis	1	2.9	7	23	6	35.3			-
S. caprae	4	11.9							-
S. chromogens							9	43	-
S. cohnii	2	5.9	1	3.3	6	35.3	1	4.7	-
S. epidermidis							2	9.5	-
S. haemolyticus	1	2.9	7	23			2	9.5	-
S. lentus	3	8.8							-
S. saprophyticus	16	47							-
S. simulans	1	2.9			1	5.9			-
S. warneri					4	23.5			-
S. xylosus	4	11.8	11	36.8			2	9.5	-
Total	34	100	28	100	17	100	21	100	

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bacteria in domestic environments (Nagase *et al.*, 2002a; Nagase *et al.*, 2002b). *Staphylococcus haemolyticus,* which has the same origin as *S. epidemidis,* was found in the air of all the abattoirs with the exception of abattoir C (Table 4.1). This species originates from dogs and other domestic animals (Glending, 2000). *Staphylococcus lentus*, found in significant levels on the udders of goats and the skins of chickens (Nagase, 2002b; Shimizu *et al.,* 2000), was present in abattoir A only, as was *Staphylococcus saprophyticus* (Table 4.1). *Staphylococcus saprophyticus* was reported to be the most often encountered agent of urinary tract infections, identified in urine of young, sexually active people (Martineau *et al.*, 2000). Euzeby (2003) reports that S. *saprophyticus* could also occur in bovine nostrils. *Staphylococcus simulans* was isolated from abattoirs A (2.9%) and C (5.9%), and is a species known to be associated with human skin (Razonable *et al.*, 2001). *Staphylococcus warneri,* also of human origin (Glending, 2000), was isolated only from abattoir C. Coagulase negative *Staphylococcus xylosus* was found in all the abattoirs except C, and has been reported by Shimizu *et al.* (2000) to be associated with the teat skin of various domestic and wild animals.

By investigating the species distribution of staphylococci in the bioerosols of the various abattoirs, it became clear that staphylococci of various sources and origins are abundant in the airborne environment. Such organisms do not only originate from humans, and should therefore not only be attributed to the food handlers. Many species may in fact originate from poor slaughtering techniques and lack of proper abattoir management. The contribution of human-borne

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staphylococcal bacteria to the bioaerosol population is, however, considerable - an issue that should be addressed through the implementation of proper quality control (QC) and good manufacturing procedures (GMP).

4.4.3 The occurrence of *Staphylococcus aureus* and its coagulase types in bioaerosols

Commonly regarded as the most pathogenic coagulase positive species, *Staphylococcus aureus* is known to originate from skin, hair and nostrils of warm-blooded animals (Lior *et al.*, 2003). *S. aureus* coagulase types have been used successfully in epidemiological investigations of food poisoning (Shimizu *et al.*, 2000), and have contributed significantly towards the understanding of the source, transmission and spread of food poisoning. This species was isolated predominantly in the bioaerosols of abattoir D (23.8%) followed by abattoir B and A (13% and 5.9% respectively). Human beings are known to disperse *S. aureus* into the air because up to 20% of healthy individuals carry this species on their bodies (Sheretz *et al.*, 2001).

Table 4.2 shows the distribution of *S. aureus* coagulase types in the air of the various abattoirs. No *S. aureus* coagulase types were isolated from the air in abattoir C. According to Table 4.2 only coagulase types I, III, VII, and VIII were isolated in the present study. Coagulase type I, known to be abundant in the environment, was isolated from both abattoirs B (50%) and D (40%). This

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Table 4.2 Coagulase types of *Staphylococcus aureus* from the deboning areas of various abattoirs

Type(s)	Abattoir A	%	Abattoir B	%	Abattoir D	%
I			2	50	2	40
III	1	50				
VII			1	25	1	20
VIII	1	50	1	25	2	40
Total	2	100	4	100	5	100

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coagulase type is capable of producing hazardous entero-toxins. *Staphylococcus aureus* coagulase types III (found only in abattoir A,) and VII (found in abattoirs B (25%) and D (20%) have been reported to be involved in food poisoning (Shimizu *et al.*, 2000). Coagulase type III is of bovine origin as reported by Nagase *et al.* (2002a) and Shimizu *et al.* (2000) whereas coagulase type VII is the most frequently encountered coagulase type in staphylococcal food poisoning (contributing to 70% of outbreaks followed by type III with 12%). The exact reason for the dominance of coagulase type VII is unclear (Shimuzi *et al.*, 2000). The remaining coagulase type (VIII) was found in the air of all the abattoirs, predominating in abattoir D (40%, Table 4.2). This coagulase type is capable of producing the potent entero-toxin B, capable of causing severe food poisoning (Ushioda *et al.*, 1981; Nagase, 2002a; Shimizu *et al.*, 2002).

Based on the data collected in this study, both TVC and staphylococci bioaerosol counts were higher in abattoir B compared to the other abattoirs. The grade B abattoir differed from the grade A, C and D abattoirs in that the deboning area did not comprise of an enclosed room, but was merely a separate area where the deboning was performed. This aspect could be suggested as the most probable reason for the observed bioaerosol counts. Properly regulated separate rooms therefore appear to be a definite advantage in leveling off the increase of undesirable airborne contaminants.

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The results have furthermore shown that airborne staphylococci originate from various environments - a fact that could be addressed through proper management and implementation of GMP and PRP programmes. The presence of airborne *Staphylococcus aureus* coagulase types capable of producing toxins hazardous to consumers as well as to food handlers, was a significant observation, as these hazardous microbiota are probable contaminants of meat products via the air. Specific recommendations that should contribute to the overall airborne quality include: 1) directing the airflow to be countercurrent to the production flow, a practice not in place in any of the sampled abattoirs; 2) keeping temperature without exception lower than 4ºC throughout the deboning period to prevent survival of micro-organisms; 3) the use of face masks to minimise the spreading of microbes from food handler's; and 4) the drafting of guidelines for red meat abattoirs to stipulate the minimum micro-organisms permitted in the air per deboning area.

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CHAPTER 5

DESCRIPTIVE AND INFERENTIAL INDICES OF RED MEAT SPOILAGE AND CONTAMINATION

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5.1 Mathematical models of vacuum-packed red meat spoilage

The prediction of the growth, survival and inactivation responses of micro-organisms to different environmental conditions has been increasingly done using predictive microbiology involving mathematical models. Predictive microbiology is a system aimed at quantitative estimation of microbial growth in foods using mathematical models (Zwietering *et al*., 1996; Walls and Scott, 1997). These mathematical models are generated by exposing micro-organisms to various conditions whereupon a database will be produced for future reference. Because micro-organisms can change and adapt during food processing and storage, predictive microbiology could play a fundamental role in establishing the contamination rate of the food, keeping such changes in consideration (Walls and Scott, 1997).

Walls and Scott (1997) report that several models have been developed for predicting microbial growth in respect of food safety. This has become possible because predictive microbiology can be used to calculate the likely number of organisms at the time the food is processed and consumed. In addition, using published data, the distribution of organisms in raw foods can be determined. However, many food systems, especially red meat which is known to be a rich, complex medium for microbial growth, are dynamic and it is therefore imperative to keep in mind that predictive models are often

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applicable only to the specific experimental design for which they were developed (Zwietering *et al.*, 1996; Forsythe, 2000; Ahmed *et al.*, 2003).

In the present study, predictive microbiology principles were applied to the data collected in chapter 2 on the growth and survival of micro-organisms on fresh vacuum-packed red meat. Linear and non-linear regression modeling was performed for the individual microbiota by means of the computer programmes SPSS (Version 8) and Sigma Plot-8 for Windows (Version 8). The equations obtained are described in Tables 5.1 and 5.2, showing the linear and non-linear equations best fitted to the various growth patterns at 5ºC and 18ºC storage under vacuum-packed conditions as well as standard errors, coefficients and R^2-values. At 5ºC storage temperature, the survival of TVC, yeasts and molds, coliforms, *Staphylococcus*, non-lactose fermenting *Enterobactericeae*, *Pseudomonas aeruginosa*, *Citrobacter* and *Shigella* were defined by sigmoidal functions. *Escherichia coli*, lactose fermenting *Enterobacteriaceae*, enterococci and *Proteus* sp. were best defined with various exponential functions (Table 5.1). At 18ºC storage temperature, Table 5.2 indicates that *Shigella*, TVC and yeasts and molds were described best by sigmoidal functions, coliforms by a logarithmic function and *Proteus* by an exponential function. The remaining organisms were fitted with wave form, hyperbola and rational functions.

The R^2 values, that represent the accuracy of the curve-fit (1 being the maximum) ranged from 0.999 to 0.624 with the 5ºC storage experiment whereas a number of fits at 18ºC could only be done with an accuracy ranging

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Table 5.1 Mathematical equations representing patterns of microbial growth during storage temperatures of vacuum-packed red meat at 5ºC

Microbes	Equations	Coefficients	S.E.	R^2
Total Viable Counts	$Y=a/(1+e^{-(x-xo)/b})$	a. 2.5×10^{8} b. 1.23×10^{-1} x_o. 4.98×10^{0}	9.55×10^{6} 9.71×10^{-1} 1.17×10^{-1}	0.999
Yeasts and Molds	$Y=a/(1+e^{-(x-xo)/b})$	a. 1.49×10^{6} b. 5.89×10^{-1} x_o. 5.1×10^{0}	9.9×10^{4} 1.45×10^{-1} 1.87×10^{-1}	0.986
Escherichia coli	$Y=a(1+x)^b$	a. 4.7×10^{2} b. 3.3×10^{0}	1.94×10^{3} 1.99×10^{0}	0.624
Coliforms	$Y=y_o+a/(1+e^{-(x-xo)/b})$	a. 4.87×10^{7} b. 7.25×10^{-1} x_o. 9.89×10^{0} yo. 6.31×10^{3}	2.57×10^{9} 4.29×10^{-1} 4.30×10^{1} 1.92×10^{4}	0.996
Enterococci	$Y=y_o+ae^{(-x/d)}\sin(2\Pi x/b+c)$	a. 1.80×10^{2} b. 9.23×10^{0} c. 4.99×10^{-1} d. 9.07×10^{7} y_o. 1.58×10^{2}	7.07×10^{1} 4.94×10^{0} 1.59×10^{0} 7.22×10^{9} 6.35×10^{1}	0.768
Staphylococci	$Y=y_o+a/(1+e^{-(x-xo)/b})$	a. 1.47×10^{6} b. 2.24×10^{-1} x_o. 6.54×10^{0} y_o. 3.53×10^{3}	3.31×10^{5} 8.99×10^{-2} 2.72×10^{-1} 1.14×10^{3}	0.999
Lactose fermenting *Enterobacte-riaceae*	$Y=y_o+a(1-e^{-bx})^c$	a. 8.06×10^{4} b. 6.99×10^{0} c. 4.34×10^{9} y_o. -3.63×10^{2}	3.65×10^{1} 1.70×10^{2} 2.09×10^{12} 2.83×10^{4}	0.694
Non-lactose fermenting *Enterobacte-riaceae*	$Y=y_o+a/(1+(x/x_o)^b)$	a. -1.17×10^{5} b. -1.18×10^{2} x_o. -5.02×10^{6} Y_o. 4.40×10^{3}	6.64×10^{4} 5.69×10^{9} 7.92×10^{5} 2.96×10^{4}	0.636
Shigella	$Y=e^{a(x-xo)}$	a. 3.80×10^{0} x_o. 5.34×10^{0}	7.59×10^{-2} 6.34×10^{-1}	0.999
Pseudomonas aeruginosa	$Y=y_o+a/(1+e^{-(x-xo)/b})$	a. 4.57×10^{3} b. 6.06×10^{-2} x_o. 4.11×10^{0} Y_o. 1.96×10^{2}	9.45×10^{2} 2.01×10^{3} 7.92×10^{5} 2.96×10^{4}	0.636
Citrobacter	$Y=a/(1+e^{-(x-xo)/b})$	a. 3.6×10^{3} b. 9.18×10^{-2} x_o. 4.06×10^{0}	1.18×10^{5} 5.99×10^{1} 9.06×10^{1}	0.632
Proteus	$Y=y_o+ae^{(-x/d)}\sin(2\Pi x/b+c)$	a. 2.13×10^{5} b. 1.99×10^{0} c. 6.13×10^{0} d. 7.59×10^{-1} y_o. 1.66×10^{3}	3.30×10^{8} 9.69×10^{3} 2.59×10^{5} 1.36×10^{3} 1.05×10^{3}	0.879

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Table 5.2. Mathematical equations representing patterns of microbial growth during storage temperatures of vacuum-packed red meat at 18°C

Microbes	Equations	Coefficients	S.E.	R^2
Total Viable Counts	$Y=y_o+a[1+e^{-((x-xo)/b)^c}]$	a. 4.04×10^{10} b. 4.94×10^{0} c. 3.88×10^{-1} x_o. 3.40×10^{2} y_o. 2.04×10^{8}	8.37×10^{20} 7.67×10^{-2} 9.06×10^{-1} 1.42×10^{-1} 6.30×10^{12}	0.998
Yeasts and Molds	$Y=yo+a/(1+e^{-(x-xo)/b})$	a. 4.75×10^{10} b. 4.04×10^{0} x_o. 3.29×10^{2} y_o. 2.53×10^{9}	2.46×10^{17} 1.16×10^{7} 1.27×10^{8} 2.35×10^{9}	0.922
Escherichia coli	$Y=a[\sin 2\Pi x/b+c]^2$	a. 8.63×10^{9} b. 6.72×10^{-1} c. $2.61 X 10^{0}$	2.73×10^{9} 1.51×10^{2} 4.48×10^{-1}	0.522
Coliforms	$Y=y_o+a\ln(x-x_0)$	a. 1.57×10^{10} x_o. -8.87×10^{2} yo. -1.08×10^{11}	2.01×10^{11} 1.32×10^{4} 1.58×10^{12}	0.302
Enterococci	$Y=a\sin 2\Pi(x-x_o/b)^2$	a. 1.37×10^{10} b. 3.12×10^{2} x_o. -2.39×10^{2}	7.22×10^{9} 1.04×10^{2} 1.43×10^{2}	0.319
Staphylococci	$Y=(1+ax)/(b+cx)$	a. $-1.37x 10^{-1}$ b. -4.95×10^{-7} c. 1.35×10^{-9}	2.07×10^{-1} 1.75×10^{-8} 1.31×10^{-8}	0.884
Lactose fermenting *Enterobacteriaceae*	$Y=ax/(b+x)+cx$	a. $-1.96x 10^{9}$ b. 8.17×10^{1} c. 1.07×10^{7}	1.47×10^{10} 9.43×10^{2} 2.73×10^{7}	0.322
Non-lactose fermenting *Enterobacteriaceae*	$Y=ax/(1+bx)$	a. 1.32×10^{6} b. -1.97×10^{-3}	9.88×10^{5} 9.08×10^{-4}	0.639
Shigella	$Y=y_o+a[1+e^{-((x-xo)/b)^c}]$	a. 5.57×10^{3} b. 2.89×10^{1} c. 2.99×10^{-1} x_o. 4.96×10^{2} Y_o. -4.39×10^{1}	1.03×10^{7} 3.1×10^{4} 3.2×10^{2} 1.8×10^{5} 6.39×10^{1}	0.995
Pseudomonas aeruginosa	$Y=ax/(b+x)$	a. 7.81×10^{7} b. 8.21×10^{1}	1.57×10^{8} 5.26×10^{2}	0.684
Citrobacter	$Y=ax/(b+x)+cx/(d+x)$	a. 8.06×10^{13} b. 5.43×10^{5} c. 2.39×10^{10} d. -4.99×10^{2}	1.6×10^{18} 1.07×10^{10} 1.17×10^{12} 3.76×10^{3}	0.266
Proteus	$Y=ax(1+x)^b$	a. 1.46×10^{3} b. 2.48×10^{0}	2.64×10^{4} 1.81×10^{0}	0.574

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from R^2=0.266 – 0.998. The accuracy of the curve-fit for the organisms *Citrobacter*, coliforms, enterococci and lactose fermenting *Enterobacteriaceae* at 5ºC were therefore not as high as for the other organisms.

5.2 Applications of predictive indices

Under specified conditions, predictive microbiology can provide estimates of the number of hazardous micro-organisms in a product at various stages during processing and/or storage. When all the variables are known, the fate of the product can be decided, based on whether the product is considered acceptable or not, and pro-active steps to minimise any risk to the consumer can be taken. The mathematical equations proposed in the present study may be applied to predict the growth of microbiota in the meat from the abattoir studied, specifically under the storage conditions reported on in chapter 2. For example, by applying the principle outlined in Figure 5.1, a X-coordinate can be calculated for a known Y-coordinate and *vice versa*. In other words, if shelf-life (time) and counts ($CFU.g^{-1}$) are represented by the axes, the age in terms of shelf-life can be calculated when the counts for a specific species are known. Alternatively, if the shelf-life of the product is known, the approximate numbers of the various microbiota can be calculated.

Considering the fact that the infective doses of a number of genera and species of pathogenic microbiota have been documented in literature, the mathematical equations should go a long way in enabling the calculation of

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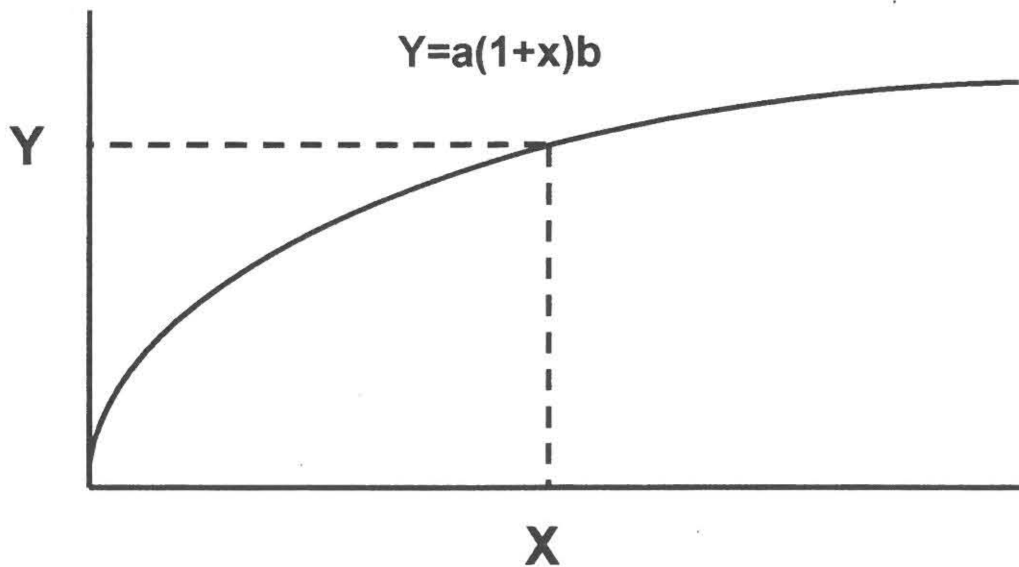

Figure 5.1 An example of application of predictive microbiology to calculate the X- or Y-coordinates for *Escherichia coli* during vacuum-packed storage at 5ºC

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the time that the product can be kept under specific conditions and still be safe for human consumption.

5.3 The contribution of staphylococci and bioaerosols to contamination of red meat in the deboning area

With reference to the aims stipulated in this study (chapter 1), the two questions that arise are the following: 1) what is the contribution of staphylococci to the total microbial population of the meat in the deboning room?; and 2) what is the contribution of the bioaerosols to the contamination of the meat in this area? To shed light on these questions, both descriptive and inferential methodologies have to be applied, as they present both visual representations as well as statistical conclusions.

Figures 5.2 and 5.3 represent visual comparisons of the extent of *Staphylococcus* occurrence compared to the Total Viable Counts in air and meat respectively. According to these graphs, the *Staphylococcus* component was the most prevalent in abattoir B and the least in Abattoir A. In the meat, however, staphylococci represented the biggest component in abattoir D with the smallest in abattoir C. Because Figures 5.2 and 5.3 are only visual representations that aid the comprehension of the predominance of staphylococci compared to the Total Viable Counts, they do not give a clear indication as to the exact relationship between the Total Viable Counts and

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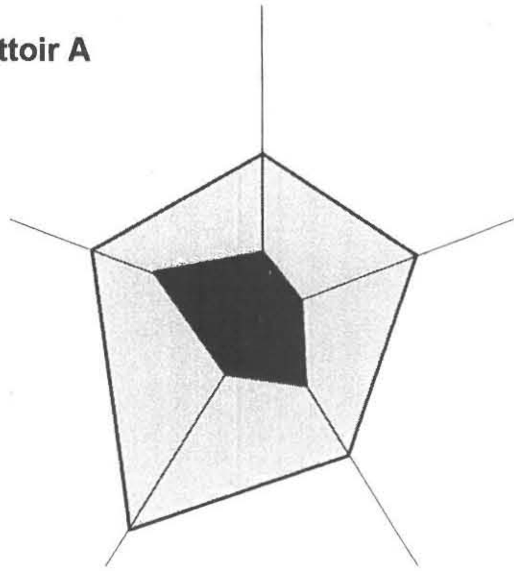
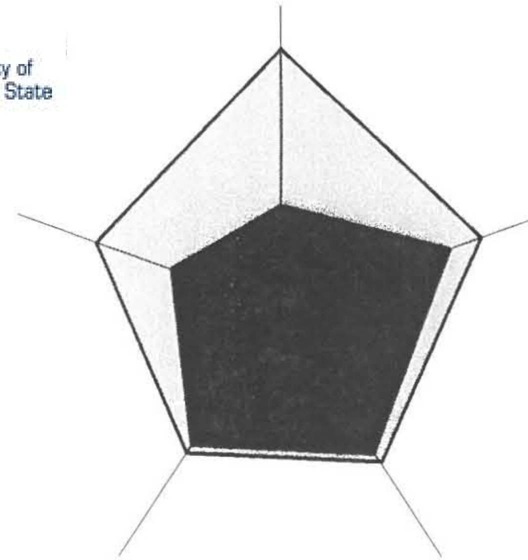
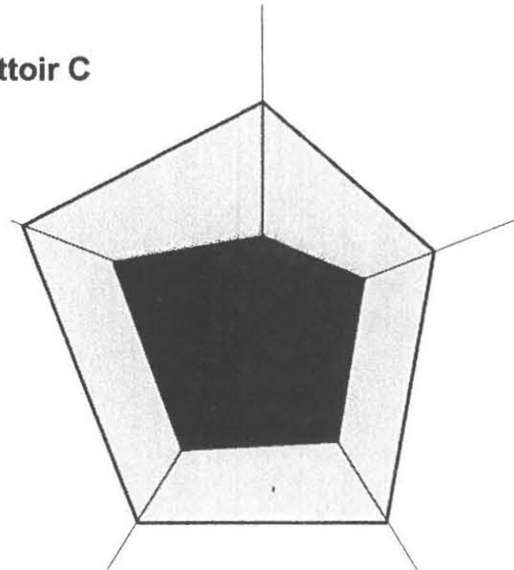
Abattoir A





Abattoir C



Abattoir D

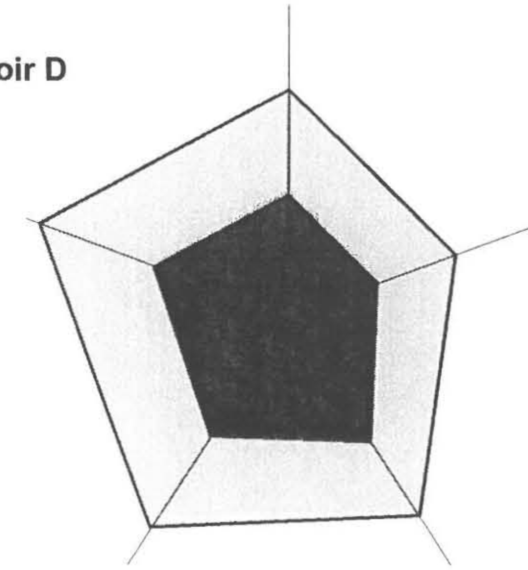


Figure 5.2 Radar plots showing the Total Viable Counts and *Staphylococcus* sp. in the air from the deboning area of red meat abattoirs over a 5-week period.

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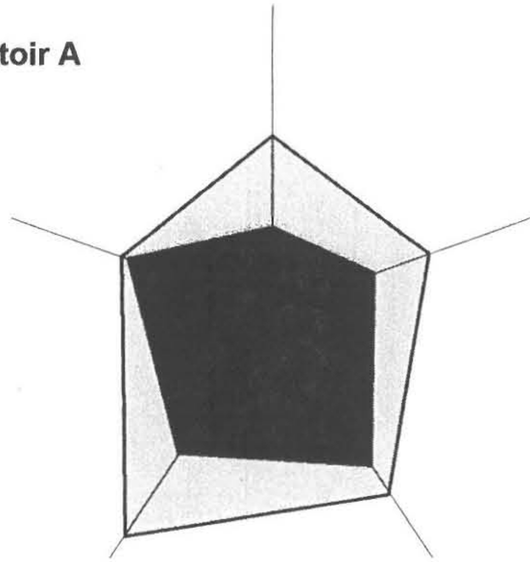
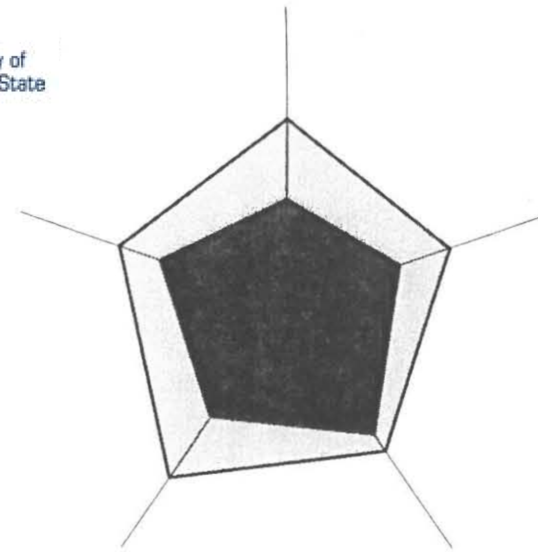
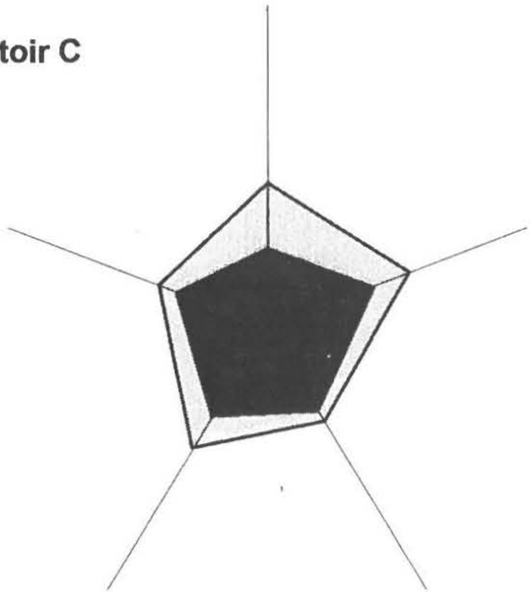
Abattoir A





Abattoir C



Abattoir D

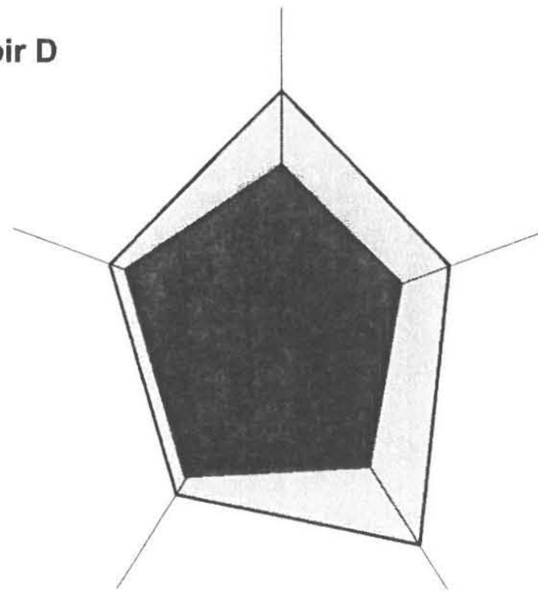


Figure 5.3 Radar plots showing the Total Viable Counts and *Staphylococcus* sp. ■ in the meat from the deboning area of red meat abattoirs over a 5-week period.

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the staphylococci during the sampling period of 5 weeks. In other words they do not indicate whether fluctuations in the staphylococci will significantly influence the total microbial composition of the air and meat. Therefore, statistical computations of such interactions are required. Table 5.3 shows the statistical correlations between Total Viable Counts and *Staphylococcus* from red meat and the air per abattoir. Interestingly, only abattoir C showed a significant correlation between the TVC and staphylococci, suggesting that there were concomitant increases and decreases amongst the numbers of these two groups of microbiota throughout the sampling period. Staphylococci thus proved to be a definite contributor to the total microbial contamination in both the air and meat at this abattoir.

Figures 5.4 and 5.5 compare the air and meat in terms of TVC and staphylococci. Although the micro-organisms are expressed per gram (meat) and per cubic meter (air) the graphs give an indication of the levels of contamination in both of these environments. Compared to abattoirs A and D, abattoirs B and C showed a larger representation of both TVC and staphylococci in the air. It therefore appeared that the level of bioaerosol contamination in the latter two abattoirs was more substantial. Table 5.4 again presents an inferential conclusion to the question regarding the relationship between the bioaerosols and the meat as the only significant correlation (r^2=0.975) was noted between the air and meat of abattoir A, in terms of the Total Viable Counts. In other words, the air considerably influenced the TVC counts on the meat in abattoir A.

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Table 5.3 Statistical correlations between total viable counts and *Staphylococcus* sp. from red meat and the air per abattoir

Grades	A	B	C	D
Meat	0	-0.231	0.959	-0.313
Air	-0.031	-0.358	0.941	-0.795

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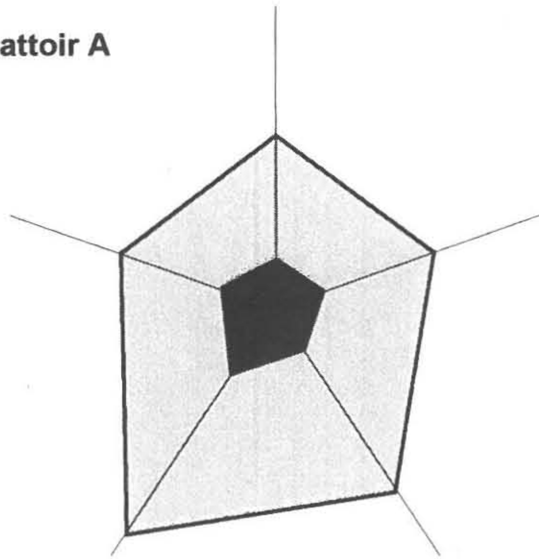

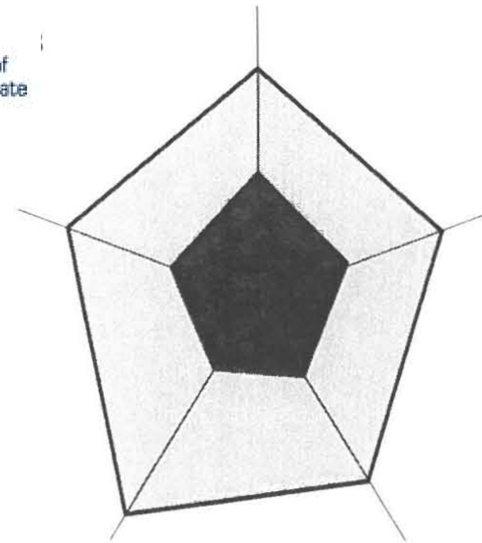

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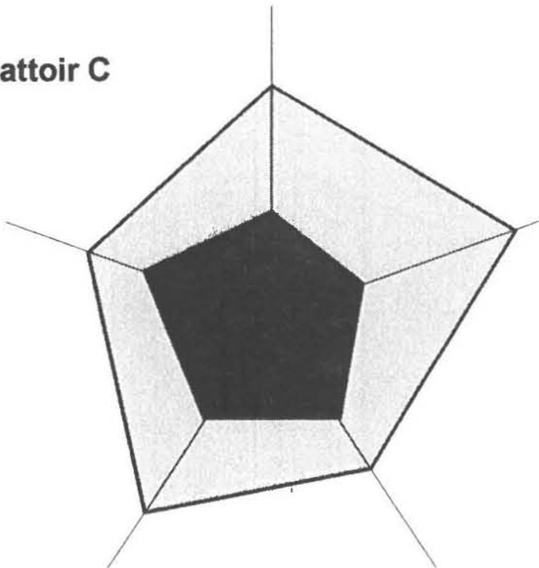

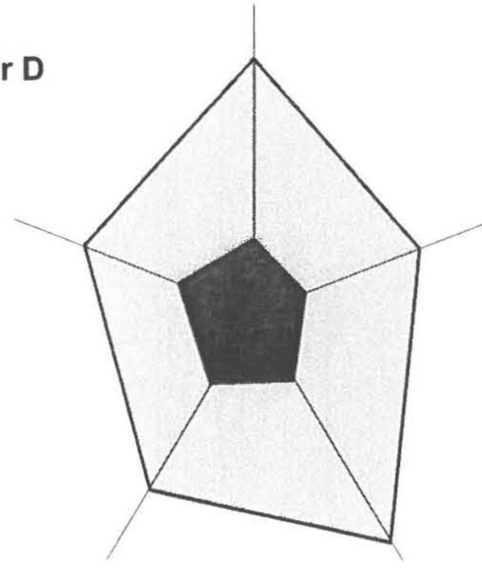

Figure 5.4 Radar plots showing the Total Viable Counts in the meat and air in the deboning area of red meat abattoirs over a 5-week period.

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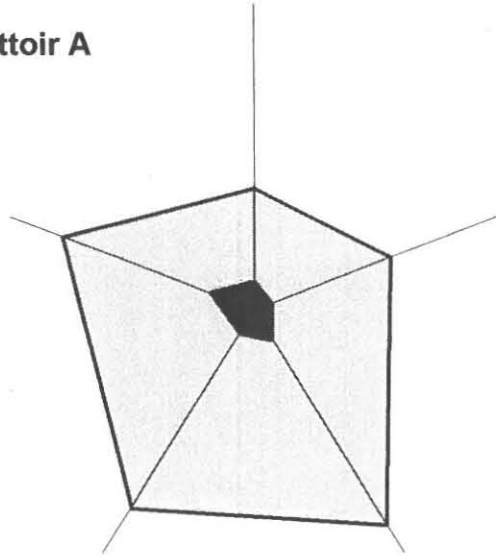
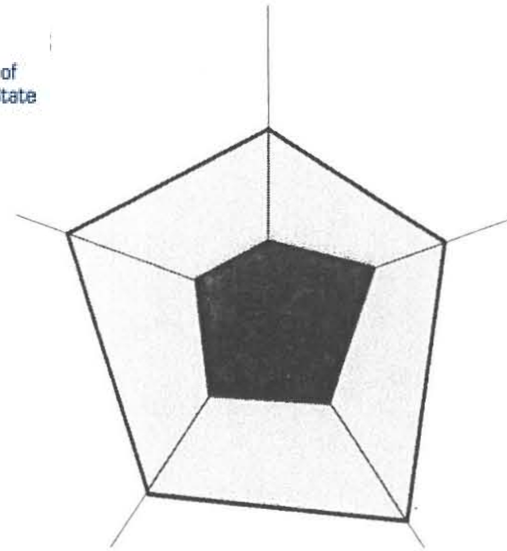
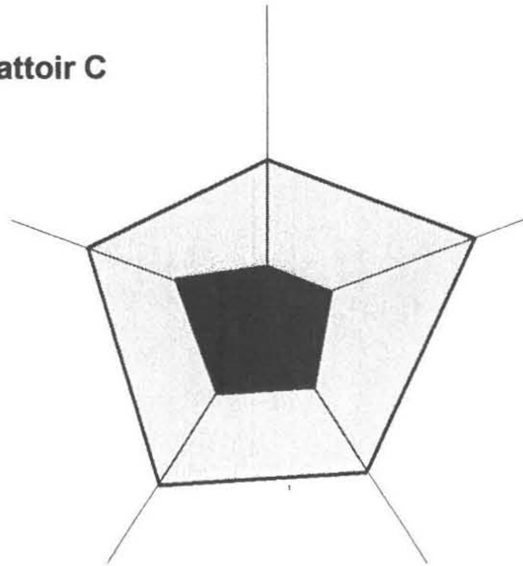
Abattoir A





Abattoir C



Abattoir D

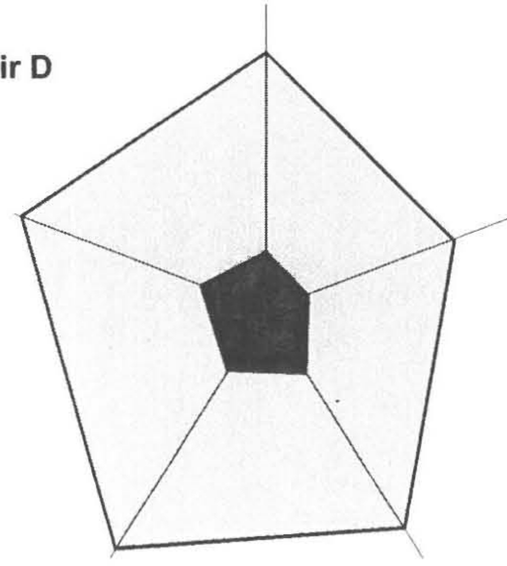


Figure 5.5 Radar plots showing the staphylococci counts in the meat and air in the deboning area of red meat abattoirs over a 5-week period.

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Table 5.4 Statistical correlations between airborne and meat-borne total viable counts and *Staphylococcus sp.* per abattoir

Grades	A	B	C	D
Staphylococcus	0.009	0.185	-0.133	0.754
Total Viable Counts	0.975	-0.413	-0.463	-0.179

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Although attempts have been made in this chapter to suggest indices of spoilage of vacuum-packed red meat as well as to apply descriptive and inferential statistics to assess the true impact of bioaerosols in the contamination of red meat with regard to the mentioned micro-biota, it became clear that the models are only applicable to an extent on the specific localities and under the experimental conditions used in this study. To extrapolate these results, a representative sample of grade A-D abattoirs will have to be investigated and a great many variables, such as slaughtering environment, infrastructure, whether free-grazing or feedlot cattle are slaughtered etc. will have to be standardised. This chapter, however, suggests new avenues in terms of the approach to predict and measure contamination of foodstuffs, and in particular red meat.

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CHAPTER 6

CONCLUSIONS

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In recent years, South African abattoirs have been increasingly looking to export their products to overseas and neighbouring countries. This has placed increased demands not only on the transport and storage conditions of fresh meat, but also on the initial microbiological quality of the meat at the time of packaging. Control of contamination in red meat abattoirs is to a considerable extent dependent on the effectiveness of Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP). Programmes such as the Hazard Analysis Critical Control Point system and its associated pre-requisite programmes (PRP's) are further required to control contamination and identify hazards. However, without in-depth information regarding the microbiota inhabiting the meat as well as on the changes that the microbial population is likely to undergo during processing and storage, management systems such as HACCP will only be effective to an extent. Information on the microbiological composition of the product should be aimed at the pathogenicity of the organism, and not only at traditional or documented indicator organisms. The fact that this study investigates the staphylococci up to species and coagulase type level, underlines the notable differences that exist within the genus with regard to its pathogenicity.

The types and numbers of microbiota that occurs in specific environments can be regarded as "fingerprints" of the fate of that specific environment. Such fingerprints can be used to predict the changes in such an environment that will influence the proliferation of the various microbiota. Likewise, the study of the microbial populations inhabiting a particular foodstuff presents ample information on its origin, processing and storage conditions. In the case of

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staphylococci, the species and coagulase types are closely linked to its origin and can therefore provide a valuable source of information pertaining to the fate of the product that it inhabits. Keeping the above mentioned in mind the overarching purpose of this study was to investigate staphylococci, their numbers, species and coagulase types as an indicator of contamination, shelf-life and processing of red meat. The investigation was expanded to include various abattoirs and focused on the deboning area where the majority of activities and carcass-handling takes place. Together with selected additional microbial groups, staphylococci were finally investigated as a variable in the establishment of possible predictive and inferential indices of red meat spoilage and contamination.

6.1 Key observations

Abattoir C which proved to be on average the least contaminated abattoir in the study was equipped with air extractors. Moreover, the movement of workers (in and out of the deboning room) was more controlled compared to the other abattoirs. It was interesting to note that, of all the abattoirs studied, this abattoir is the only that processes both pork and lamb simultaneously with bovine. This explains why some species of staphylococci originating from animals other than cattle were isolated. Abattoir A, the highest throughput abattoir, was on average the second least contaminated. The bioaerosol counts noted may in particular be attributed to improper ventilation because of the absence of air extractors at this abattoir. The temperature at which meat handler's worked (4°C) in the deboning room of abattoir A led to workers

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singing, jumping, talking and moving frequently to keep themselves warm. If not managed properly, there could thus be a serious down-side to maintaining extremely low temperatures in the deboning room and expecting food handler's to work for extended periods in such conditions.

Although both the above mentioned abattoirs debone meat in closed rooms with abattoir C processing the product at 10ºC, the difference in temperature at which deboning is performed do not appear to directly contribute to the survival of microbiota in the air and on the meat. Abattoirs B and D debone meat in an open deboning room at ambient temperature and consumers are allowed to enter and choose the meat they preferred. A lack of proper facilities, together with poor standards in the deboning rooms which lacked ventilation systems, air extractors and hand washing facilities, could further be a definite contributor to the elevated meat and bioaerosol counts in these abattoirs. Abattoir D makes use of a butchery combined with a supermarket for deboning thereby exposing the product to a whole host of external contaminants.

6.2 Recommendations to industry

In general, airborne microbiota gets transmitted to the deboning room with carcasses brought in from cold rooms as well as from biofilms settling on surfaces such as conveyer belts and working tables. When the meat is exposed to these biofilms, it becomes recontaminated and the friction that is

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encountered during processing aerosolise the microbiota. Contamination of the meat from bioaerosols should not be neglected as there is a definite possibility that some of the more notorious strains (for example *Staphylococcus areus*, Coagulase Type VIII) might be introduced via the air.

In overcoming the problems stated above, the new draft legislation on the grading of red meat abattoirs (Table 1.2, chapter 1) should go a long way in ensuring quality and safety as it enforces the involvement of Environmental Health Practitioners to monitor red meat abattoirs on the basis of good manufacturing practices (RSA, Department of Health, 2000; RSA, National Department of Agriculture, 2003). Under this new system, it is suggested that abattoirs be graded based on the quality of their products rather than infrastructure or throughput. The draft legislation furthermore states that a laboratory should be at hand to conduct microbiological analysis. Additional legislation has recently been implemented that requires the implementation of HACCP in all food premises that produces food products for export (Ramphal, 2004).

In addition to the mentioned recommendations, red meat abattoirs should consider the application of environmental sanitizers such as ammonia or chlorine-based chemicals to reduce the levels of staphylococci in the air. Along with these sanitizers, humidity levels should be kept as low as possible to avoid distribution of bioaerosols. The use of facial masks should be enforced to circumvent spreading of oral staphylococci. Because of the ubiquitous occurrence of staphylococci, spraying the cattle with water and/or

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organic sprays to reduce microbial contaminants on the animal's skin prior to slaughtering should be investigated. During the skinning and evisceration processes the carcasses are exposed to a multitude of contaminants from the skin and gut and should therefore be washed with acetic or lactic acid solutions prior to chilling and not only with water to remove the visible dirt. Both the above mentioned methodologies have been applied successfully locally as well as in many abattoirs around the world. However, at the abattoirs sampled during the extent of this study, these methodologies have either been applied very ineptly or not at all.

In the deboning room, it is crucial that the surfaces be disinfected with organic acids sprays. The use of alcohol-based hand-rubs should be implemented in all abattoirs – a system applied only in abattoir A at the time of this study. The choice of sanitising and detergent agents is of paramount importance, as it is known that staphylococci is becoming increasingly resistant to quaternary-ammonia based chemicals. In addition, the equipment (gloves, knives and hooks) used for deboning should be disinfected frequently – preferably between handling each carcass. Workers should be checked for any visible signs of cuts or bruises on there hands and arms and they should be encouraged to report these, irrespective of how minor they may seem, to the resident nurse.

Although slaughtering of more than one species does not appear to have a significant influence on the numbers of contaminating organisms at, for example abattoir C, it is the types of strains that can be transferred from one

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carcass to the other that is a reason for concern. With the aim of avoiding cross contamination during slaughtering of more than one animal species (bovine, sheep, pigs etc.), their separation during slaughtering should be a priority. If specialization of abattoirs in this regard is not possible, tangible measures should be put in place to keep the processing lines of each species separate as far as possible. Finally, similar to the requirements for the successful implementation of management systems such as HACCP and ISO 9000, it is imperative that abattoir management takes ownership of the safety and quality of the products produced, as many of the problems with regard to meat safety can be overcome through practicing proper and sound management principles.

6.3 Future research

As a direct result from this study, the following were identified as possible future research projects:

- A study into the prevalence of staphylococci and other microbiota associated with feedlot cattle as apposed to free-grazing animals. Such a study will provide valuable information to both industry and consumers on the quality and wholesomeness of products from these two groups;
- The expansion of the study to include species other than bovine;

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- Expansion of the sample to aid the applicability of predictive microbiology on a wider front;
- A study into the occurrence of staphylococcal enterotoxins in meat and other food products compared to the levels of the organism in the actual product;
- A comparative study on the analytical procedures used to isolate and identify staphylococci. Such methodologies would include the comparison of *Staphylococcus aureus* coagulase typing with plasmid profiling, plasmid and chromosomal macro-restriction, ribo-typing, DNA hybridization, PCR assays, ELISA techniques and pulsed field gel electrophoresis.

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6.4 References

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APPENDIX

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Figure 5.6 Illustration of a typical deboning process

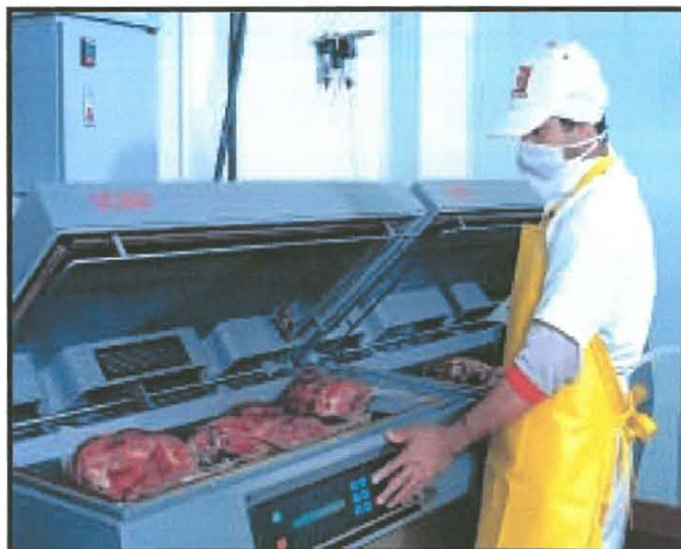

Figure 5.7 An example of a vacuum-packaging machine used in a deboning plant

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Figure 5.8 The SAS Super 90 microbial air sampler during sampling at the deboning room

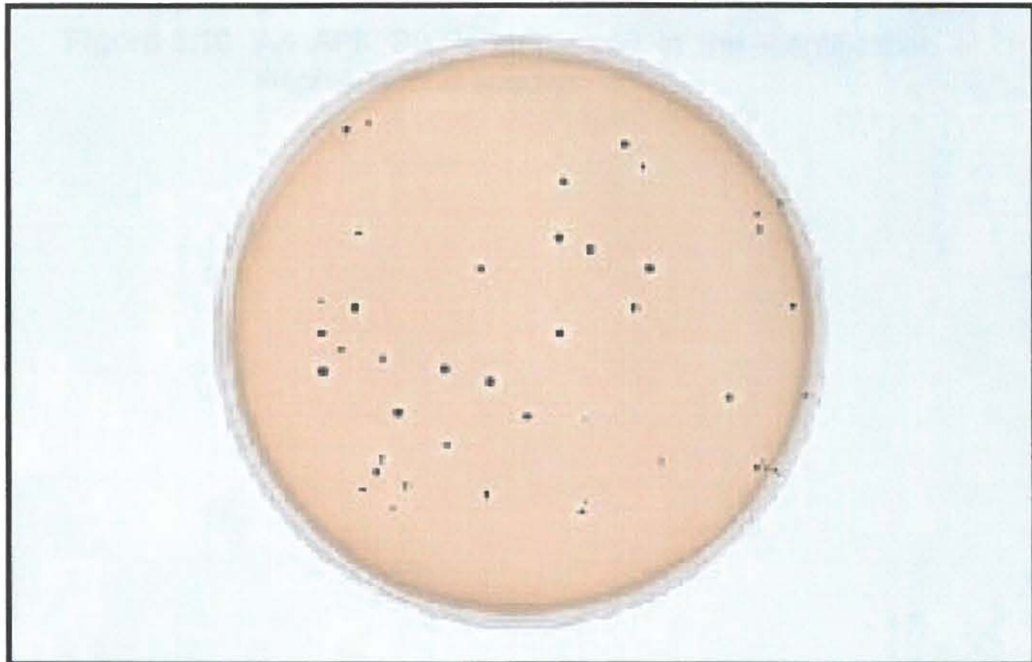

Figure 5.9 *Staphylococcus* colonies on Baird-Parker Agar

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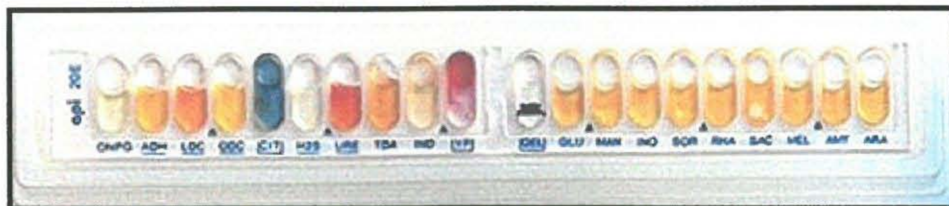

Figure 5.10 An API- Staph strip used in the identification of staphylococcal species

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