THE OCCURRENCE OF *STAPHYLOCOCCUS* SPECIES IN THE DEBONING ROOM OF A HIGH-THROUGHPUT ABATTOIR

by

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DECLARATION OF INDEPENDENT WORK

I, ZELDA PLAATJIES, do hereby declare that this research project submitted to the Central University of Technology for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH, is my own independent 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.

Z Plaatjies ..... 12 April 2004

SIGNATURE OF STUDENT DATE

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SUMMARY

THE OCCURRENCE OF *STAPHYLOCOCCUS* SPECIES IN THE DEBONING ROOM OF A HIGH THROUGHPUT ABATTOIR

Meat and meat products are highly perishable foods that frequently present problems with regard to staphylococcal food poisoning because of the considerable handling of the foodstuff that takes place during preparation. Due to the ubiquitous nature of *Staphylococcus* information pertaining to the prevalence of staphylococci on meat can shed light on the sources of contamination as well as the level of worker and process hygiene.

Red meat samples were collected from the deboning room of a high throughput abattoir and exposed to storage temperatures of 5°C and 18°C respectively. During the entire shelf-life study, the total viable counts and *Staphylococcus* levels remained approximately 60% lower at 5°C than at 18°C. Throughout the shelf-life experiment, the staphylococci counts exceeded the national guideline of 100 $CFU.g^{-1}$ for meat during exposure to both temperatures with staphylococcal counts peaking at 10^5 $CFU.g^{-1}$ at 18°C. Red meat samples prior to vacuum-packaging (directly from the conveyor-belt) as well as bioaerosol samples were furthermore collected and analysed for total viable counts and the presence of *Staphylococcus* species. The meat was found to be below the microbiological guidelines for raw meat as proposed by the South African Department of Health. The presence of airborne staphylococci counts was 38% compared to 62% in the sampled

meat. The total viable counts from workers' hands and working surfaces were relatively high and well above the national guideline of 100 CFU.cm^{-2} for working surfaces. The mean staphylococci counts from the surfaces were 19 CFU.cm^{-2} and these surfaces were found to be moderately contaminated as the levels were above 10 CFU.cm^{-2}. The following *Staphylococcus* species were isolated throughout the study: *Staphylococcus aureus; S. epidermidis; S. capitis; S. auricularis; S. hominis; S. saprophyticus; S. haemolyticus; S. simulans; S. sciuri; S. intermedius; S. xylosus; S. cohnii cohnii; S. lugdunensis* and *S. warneri.*

The presence of the above-mentioned staphylococci points to direct and indirect contamination of meat through, amongst others, the meat handlers in the deboning room as the majority of the species are associated with humans. It was deduced that the meat handlers needed to be educated on the importance of proper, safe hygienic working practices as thirteen of the above identified species are toxin-producers. These toxins are predominantly heat stable and are likely to endure the heating process. Because of the adherence ability of this organism special attention should be given to the cleaning and sanitation programmes of the deboning room, especially since some of the *Staphylococcus* species have been found to be resistant against quaternary ammonium-based cleaning agents.

OPSOMMING

DIE VOORKOMS VAN *STAPHYLOCOCCUS* SPECIES IN DIE ONTBENINGSLOKAAL VAN 'N HOË-DEURSET ABATTOIR

Vleis en vleisprodukte is hoogs-bederfbare voedsels en dikwels 'n probleem in terme van staphylokokkale voedselvergiftiging omdat die produk onderhewig is aan 'n groot mate van hantering gedurende prosessering. Staphylokokke kom voor in die lug, stof, water, riool, melk, voedsel en voedseltoerusting, omgewingsoppervlaktes asook mense en diere. Inligting rakende die voorkoms van staphylokokke op vleis kan lig werp op die bronne van kontaminasie, en die vlak van werker en proses higiëne.

In hierdie studie is rooivleismonsters vanaf 'n ontbeningslokaal van 'n hoë-deurset abattoir versamel en blootgestel aan bergingstemperature van onderskeidelik 5°C en 18°C. Tydens die totale rakleeftyd-periode was die totale plaattellings en *Staphylococcus* ongeveer 60% laer by 5°C as dié by 18°C. Die *Staphylococcus* tellings het gedurende die totale rakleeftydseksperiment die nasionale riglyne van 100 CFU.g^{-1} tydens die blootstelling aan beide temperature oorskry met maksimum tellings hoër as 10^5 by 18°C. Die totale plaattellings en staphylokokke wat in die lug voorkom en dié op die vleis voor vakuumverpakking is verder geanaliseer. In hierdie geval het *Staphylococcus* tellings nie die aanbevole mikrobiologiese wetgewing, soos voorgestel deur die Suid-Afrikaanse Departement van Gesondheid vir rou vleis, oorskry nie. Die staphylokokkale bioaërosol tellings

was 38% in vergelyking met 62% in die vleis. Die totale plaattellings verkry van die werkers se hande en die werksoppervlaktes was relatief hoog en was sonder uitsondering bo die nasionale riglyn van 100 $CFU.cm^{-2}$ vir werksoppervlaktes. Die gemiddelde tellings van *Staphylococcus* op die oppervlaktes was 19 $CFU.cm^{-2}$ en was dus redelik gekontamineerd aangesien die tellings bo 10 $CFU.cm^{-2}$ was. Die volgende *Staphylococcus* spesies is tydens die studie geïdentifiseer: *Staphylococcus aureus; S. epidermidis; S. capitis; S. auricularis; S. hominis; S. saprophyticus; S. haemolyticus; S. simulans; S. sciuri; S. intermedius; S. xylosus; S. cohnii cohnii; S. lugdunensis* en *S. warneri.*

Die aanwesigheid van bogenoemde spesies verwys na direkte en indirekte kontaminasie van vleis deur, ondere andere, die vleishanteerders wat in die ontbeningslokaal werk, aangesien die meeste geïdentifiseerde spesies met die mens geassosieer word. Die gevolgtrekking wat gemaak is, is dat die vleishanteerders opleiding nodig het rakende die belangrikheid van geskikte en veilige higiëniese werkspraktye, aangesien dertien van die geïdentifiseerde spesies toksiene kan produseer. Hierdie toksiene is oorwegend hitte-stabiel en sal waarskynlik die verhittings proses weerstaan. Weens die vermoë van hierdie organisme om aan oppervlaktes te kleef, moet spesiale aandag geskenk word aan die skoonmaak en sanitasie prosedure in die ontbeningslokaal, veral omdat sekere *Staphylococcus* spesies bestand is teen skoonmaakmiddels met kwaternêre-ammoniak verbindings as basis.

Central University of Technology, Free State

TABLE OF CONTENTS

CHAPTER 3

THE ETIOLOGY OF STAPHYLOCOCCUS SPECIES ASSOCIATED WITH THE DEBONING ROOM OF A HIGH THROUGHPUT ABATTOIR

CHAPTER 4

THE PREVALENCE OF STAPHYLOCOCCI ON SURFACES IN THE DEBONING ROOM OF A HIGH THROUGHPUT RED MEAT ABATTOIR

CHAPTER 5

CONCLUSION

Page

LIST OF TABLES

Page

LIST OF FIGURES

APPENDIX A

CHAPTER 1

INTRODUCTION

1.1 BACKDROP TO THE SOUTH AFRICAN RED MEAT INDUSTRY

Since the deregulation of the South African red meat industry in 1993, rapid growth in the number of registered abattoirs has occurred: from a total of 330 in 1993, the number has grown to 504 in 2001 (SAMIC, 2003). The total slaughter capacity at registered abattoirs is currently estimated at 16 500 slaughter units per day (one slaughter unit equals either one head of cattle, five pigs or 15 head of sheep). It is also estimated that abattoirs nationally operate at about 60% of capacity. During the era when abattoirs were regulated, this sector largely offered only a slaughter service. However, the rapid expansion in the cattle and beef industry as well as its highly decentralised nature, has led to increased concerns about the safety of beef because processing and marketing sectors have identified unique food safety problems (Brown, Longworth and Waldron, 2002).

1.2 HYGIENE ASSESSMENT SCHEMES AND GRADING OF RED MEAT ABATTOIRS

In the meat industry hygiene means the prevention of contamination of the product; an abattoir thus has to be a food factory where the primary aim is to produce a healthy, wholesome and clean product, which is safe for consumption. It therefore needs to be a well-designed, well-constructed structure in order to efficiently cope with the slaughtering of the animal. Because there are various processes involved in production, there is a high risk of contamination of the product. The prevention of contamination of the

product is determined by the layout and flow patterns followed by the product (RSA, 2000a). Figure 1.1 shows a flow diagram of the processes involved in a typical red meat abattoir. The flow diagram identifies potential sites of minor or major contamination in the process, so that critical control points (CCPs) can be established along the process and production line.

An Abattoir Hygiene Rating Scheme aimed at improving the standards of abattoirs to above the minimum standards prescribed by the Meat Safety Act of 2000 was introduced in the Gauteng Province of South Africa during 2003 (RSA, 2000b). This scientifically-based system is used to determine objectively the hygiene and animal welfare status of all registered abattoirs (South African Red Meat Industries Companies SAMIC), 2003 (Table1.1).

All abattoirs must comply with statutory grading requirements and because a higher throughput increases the risk of contamination, the higher the grade of the abattoir, the higher the requirements (RSA, 2000a). The system further empowers the consumer to ascertain from their butchers and restaurateurs the star grading of the abattoir from which the meat originated. There are at least five grades of abattoirs, depending on the number of units slaughtered per day. The throughput thus determines the basis for the grade of the abattoir. Table 1.2 indicates the basis according to which the grading of abattoirs is regulated.

The Grade A abattoir, or high throughput abattoir, has the largest slaughtering frequency of 100 units per day. The abattoir must be fenced,

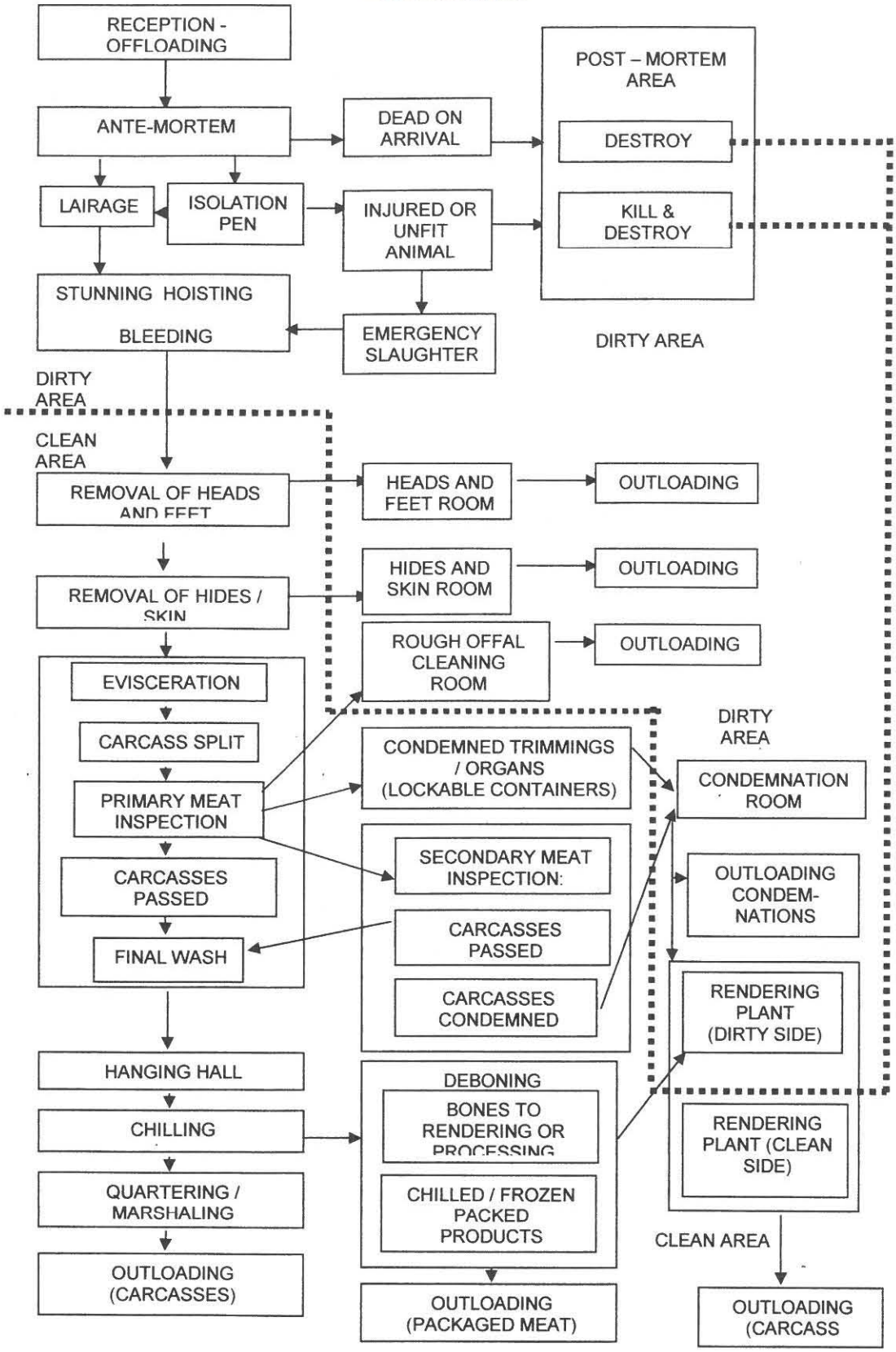

Figure 1.1 Flow diagram of a typical red meat abattoir

Table 1.1 The Hygiene Assessment System (HAS) star grading scheme used to audit abattoirs in South Africa (adapted from SAMIC, 2003)

Star Rating	Hygiene Assessment System Score[1]
★★★★★	90+
★★★★	75-89
★★★	60-74
-	46-60

1: Maximum score 100

Table 1.2 The basis according to which abattoirs are graded (adapted from SAMIC, 2003)

GRADES	SLAUGHTER UNITS[1]
A	< 100
B	51 - 100
C	16 - 50
D	9 - 15
E	1 - 8

1: 1 Slaughter unit = 1 bovine/ 1 horse/ 15 pigs/ 15 sheep

with dust and mud free roads. There have to be facilities for offloading of animals and roofed lairages, as well as adequate facilities for effective and humane stunning and bleeding. A sufficiently large slaughter hall should be built to international standards and adequate facilities for hygienic dressing of hanging carcasses and for cleaning offal. There should be a definite separation between dirty and clean areas of the abattoir as well as separation of workers authorised to work in clean areas from those working in dirty areas. Separate rooms must furthermore be available for handling of detained and condemned material.

Provision should be made for adequate facilities in terms of changing rooms, for showering and washing for all workers, as well as for truck washing facilities for livestock delivery vehicles. Most importantly there should be facilities to accommodate the meat inspection staff and finally, the boning and packing room should be covered and should boast a paved despatch area with adequate refrigeration space. As the correct infrastructure would have an effect on the quality of the product being produced, all abattoirs should have a slaughter hall which is separated by a 30cm wall from the rough offal emptying and cleaning section with a meat inspection service (Veterinary Public Health (VPH), 2003). A Grade B abattoir slaughters between five and 100 units per day and has a meat inspector who is employed by government or local authority. A Grade C abattoir slaughters not more than five units per day while meat inspection is handled by independent meat inspectors that perform inspection on an occasional basis.

1.3 THE DEBONING PROCESS AT A RED MEAT ABATTOIR

The carcass-breaking processes at large beef-packaging plants are complex and usually commence with the removing of portions from hanging sides, with each portion being assigned to one or more production lines for further processing. In any such process, the operations and equipment are difficult to examine simultaneously for sources of bacterial contamination. According to Gill, McGinnis and Bryant (1998) the numbers of bacteria on beef tend to increase during the process of breaking down of carcasses.

During breaking, the hanging carcass side is extensively handled and also comes into contact with various items of hand-held equipment. Such contact may result in contamination by bacteria from hands or equipment (Gill and Jones, 1999). Nortjé *et al.* (1990) state that much of this contamination is of faecal origin and includes staphylococci, micrococci, pseudomonads, yeasts and moulds. Many of these organisms are psychrotrophs and are able to grow at refrigerated temperatures; they are thus potential spoilage agents of chilled meat.

Unhygienic treatment of food should be considered a major risk of contamination, as staphylococcal food poisoning is often associated with manually handled food (Atanassova, Meindl and Ring, 2001). During meat processing additional sources of contamination by strains of the genus *Staphylococcus* are, amongst others, contaminated hides, faeces, the contents of the digestive organs and contaminated water. Schlegelová *et al*

(2004) report that during meat processing, horizontal contamination of meat may occur, which may be highly significant in some cases. The authors report that 92-100% of minced beef was found to be contaminated with *S. aureus*. Horizontal contamination is when the product is exposed to contact surfaces such as conveyor belts, stainless steel tables and handling equipment and the product is contaminated by the accumulation of debris from inadequate sanitation. The opportunity for contamination of the meat therefore exists, amongst others, from the slaughter floor, throughout the production chain to the retailer, through contact with surfaces and through handling. Therefore it is important that a food plant possesses a schematic layout of the production process so that possible sources of contamination can be identified. Figure 1.2 is a schematic representation showing the deboning process at a typical red meat abattoir.

1.4 BIOLOGICAL HAZARDS IN THE SLAUGHTERING PROCESS OF RED MEAT

Throughout the world, meat inspecting authorities either encourage or require meat packaging plants to implement Hazard Analysis Critical Control Point (HACCP) systems for their processes. The Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA) has been in the forefront of current efforts to improve the general microbiological safety of meats and raw beef in particular. This was done through the implementation of HACCP systems for meat plant processes in order to minimise the potential microbiological hazards within this industry (Brown, Longworth and

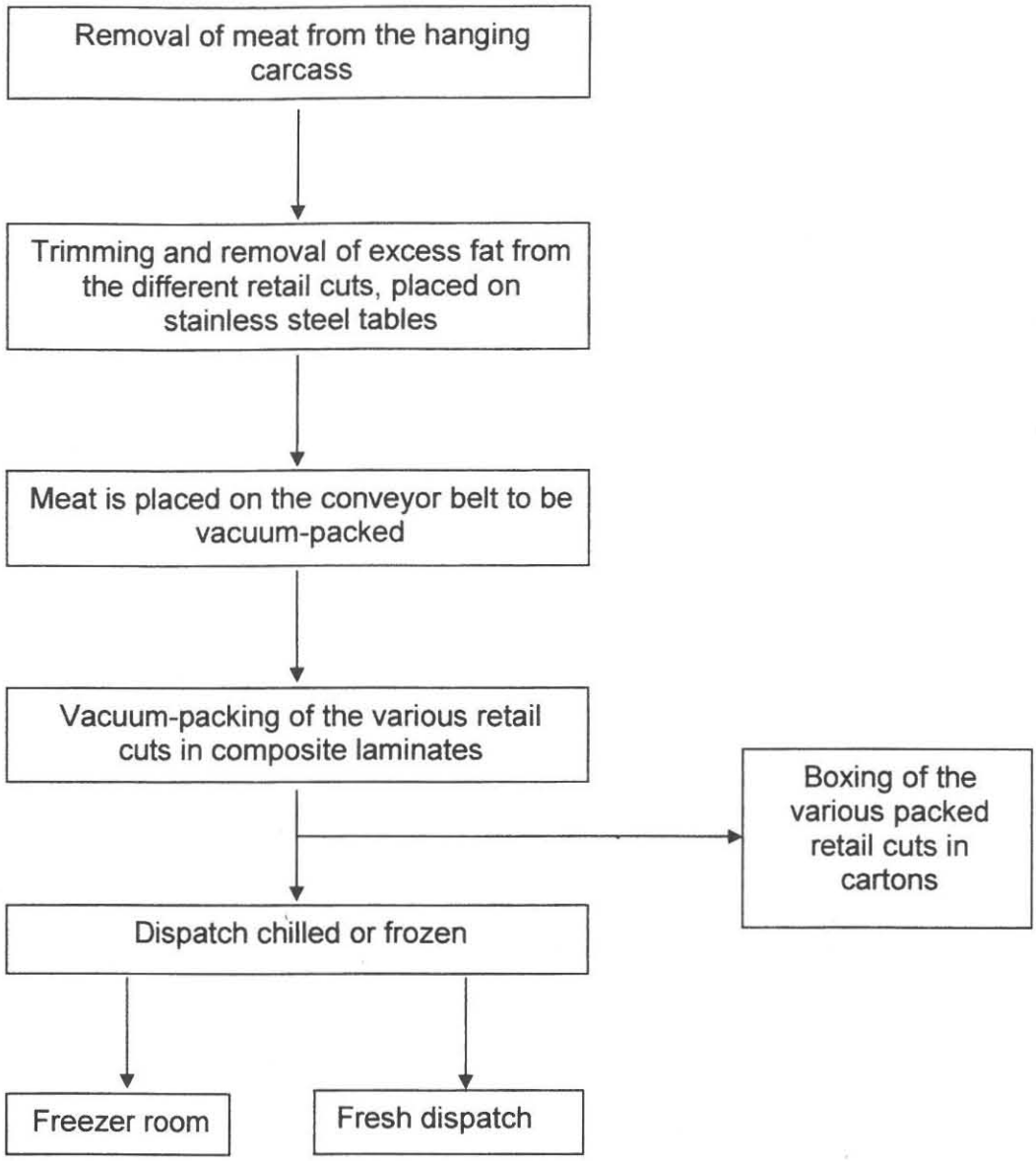

Figure 1.2 Schematic representation of the deboning process at a red meat abattoir (adapted from Nel, 2003)

Waldron, 2000). In South Africa however, many abattoirs are still in the beginning stages of the implementation of a HACCP system. Improving the microbiological quality of foods alone is insufficient especially when food-processing technologies cannot always guarantee the absence of pathogens in foods easily becoming re-contaminated. Furthermore it was also found that foods of animal origin are the foods most often involved in disease outbreaks, with red meat being the predominant vehicle in foodborne disease outbreaks (Panisello *et al.*, 2002; Reid *et al.*, 2002).

Numerous factors interact to affect the level of hide contamination on animals presented for slaughter, which can then affect the microbiological content of the carcass. The degree of visible contamination on the hide has been shown to affect the degree of subsequent contamination of the carcass. However, a visibly clean hide may not necessarily be pathogen-free and may still present a potential hazard for cross-contamination of the resulting carcass (McEvoy *et al.*, 2000; Buncic *et al.*, 2002). Because it has long been recognised that the slaughter stock is a major cause of carcass contamination, the United Kingdom has developed a 5-point scoring system (Meat Hygiene Service) to assess all cattle on visible cleanliness before slaughter. In Table 1.3 the 5-point scoring system to assess the cleanliness of carcasses before slaughter is shown. Lower scores (1–2) are given to visibly clean/dry animals and the higher scores (4–5) given to excessively dirty (mud, faeces) and wet animals. The latter groups are not permitted to be slaughtered as they are considered to pose a high risk of carcass contamination during dressing (Reid *et al.*, 2002). Bell (1997) reports that the beef slaughtering and dressing process,

Table 1.3 The 5-point scoring system to assess the cleanliness of carcasses before slaughter (adapted from Reid *et al.*, 2002)

Hygiene Assessment System (HAS)	
Cleanliness	**Score**
Visibly clean / dry animals	1 –2 (low)
Excessively dirty (mud, faeces)	4 – 5 (High)

the holding pens and the carcass skinning and evisceration processes are probable introduction points of major contamination. It has been demonstrated that bacterial contamination of edible tissues, via blood circulation, can occur if a contaminated knife is used for cutting during slaughtering operations (Buncic *et al.*, 2002).

Two major sources of bacteria in meat and meat products have been identified. Both sources are harboured by the living animal, thus explaining the presence of these pathogenic bacteria in the processing environment. In addition humans are also prominent sources of pathogenic bacteria, most frequently by indirect cross-contamination. The majority of the pathogens that the food industry contends with today are not newcomers to the meat industry. For example *Salmonella, Staphylococcus aureus, Bacillus cereus* and *Clostridium botulinum* have been associated with foodborne illness for decades and it is well documented that *Listeria* spp., *S. aureus, Yersinia enterocolitica, Salmonella* and *Aeromonas* spp. may be detected in the slaughterhouse environment, for instance on the floor and walls, on cold room floors, hand basins, splitting saws and chopping blocks (Borch and Arinder, 2002). Various studies have reported on the above-mentioned microorganisms, especially *Escherichia coli* (McEvoy *et al.*, 2000; McEvoy *et al.*, 2003; Sumner *et al.*, 2003). It is furthermore generally acknowledged that traditional meat inspection procedures cannot always ensure that consumers will not be exposed to infectious doses originating from meat-borne pathogens. Panisello *et al.* (2002) note the contributing factors that lead to general foodborne outbreaks (Table 1.4).

Table 1.4 Contributing factors to general foodborne outbreaks (adapted from Panisello *et al* (2002)

Contributing Factors	Outbreaks[1]	
	No.	**(%)**
Temperature misuse		
Improper heating	211	39.8
Improper reheating	14	2.6
Inadequate storage	170	32.1
Preparation too far in advance	15	2.8
Inadequate thawing	10	1.9
Inadequate Handling		
Food Handler	48	9.1
Cross-contamination	118	22.3
Inadequate Environment		
Insufficient hygiene	15	2.8
Inadequate facilities	5	0.9
Raw material		
Raw ingredient	116	21.9
Infected animals	3	0.6

1: Percentages are based on total number of outbreaks (n = 530)

1.5 STAPHYLOCOCCI AS FOODBORNE PATHOGENS

Holt *et al.* (1994) summarised staphylococci as having spherical cells of 0,5–1,5 µm in diameter that occur either singularly, in duo, or in irregular clusters. *Staphylococcus* bacteria are non-sporing, non-motile facultative anaerobes which are also Gram-positive organisms. The colonies are non-transparent and can be white, cream and occasionally yellow to orange in colour, with an optimum growth temperature of 30–37°C. Staphylococci are ubiquitous in nature and the presence of genera such as *S. aureus* in food products is dangerous, as a number of strains cause foodborne intoxication. Intoxication occurs when food is ingested in which enterotoxigenic strains have grown to sufficient levels to permit a toxic dose of staphylococcal enterotoxin (SE) to be produced prior to consumption (Desmarchelier *et al.*, 1999; Vanderlinde *et al.*; 1999; Borch *et al.*, 2002,). Staphylococci are usually associated with the skin and mucous membranes of warm-blooded vertebrates but are often isolated from food products, dust and water (Holt *et al.*, 1994). The genus *Staphylococcus* includes over 30 species and those of potential interest are listed in Table 1.5. Of the 18 species and subspecies noted in the table, 6 are coagulase-positive and produce thermostable nuclease, while 10 of the coagulase-negative species have been shown to produce enterotoxins.

Jay (2000) states that the long-standing practice of examining foods for coagulase-positive staphylococci as strains of importance has undoubtedly led to underestimation of the prevalence of enterotoxin producers.

Table 1.5 Staphylococcal species and subspecies known to produce coagualse, nuclease, and/or enterotoxins (adapted from Jay, 2000)

Organisms	Coagulase	Nuclease	Enterotoxin
***Staphylococcus aureus* subsp**			
S. naerobius	+	TS	-
S. aureus	+	TS	+
S. intermedius	+	TS	+
S. hyicus	(+)	TS	+
S. delphini	+	-	
***S. schleiferi* subsp.**			
S. coagulans	+	TS	
S. schleiferi	-	TS	
S. caprae	-	TS	+
S. chromogens	-	-w	+
S. cohnii	-	-	+
S. epidermidis	-	-	+
S. haemolyticus	-	TL	+
S. lentus	-		+
S. saprophyticus	-	-	+
S. sciuri	-		+
S. simulans	-	v	
S. warneri	-	TL	+
S. xylosus	-	-	+

Note: + = positive; - = negative; -w = negative to weakly positive; (+) = weak reaction; v = variable; TS = thermostable; TL = thermolabile

Furthermore Jay (2000) reports that among the coagulase–positive species, *S. intermedius* is a well-known enterotoxin producer.

A number of the *Staphylococcus* species are host–adapted, with about 50% of the known species inhabiting humans only (for example *S. cohnii* subsp. *cohnii)* while *S. aureus* inhabits humans and other animals. The largest numbers tend to be near body orifices such as anterior nares, axillae and the inguinal and perineal areas. In moist habitats the organisms may reach numbers per square centimetre of $10^3 - 10^6$, and in dry habitats, $10 - 10^3$. The two primary sources of contamination in foods are nasal carriers and food handlers whose arms and hands are infected with boils, sores or wounds, and who are permitted to handle food.

Leading factors that have given rise to the outbreak of staphylococcal foodborne gastroenteritis are improper holding temperatures, poor personal hygiene, contaminated equipment, inadequate cooking and foods from unsafe sources (Jay, 2000). *Staphylococcus aureus* has been considered to be the second or third most common pathogen to cause outbreaks of food poisoning in many countries and is still only outnumbered by outbreaks from *Salmonella* spp. and *Clostridium perfringens*.

1.6 RATIONALE

According to Smulders and Greer (1998) considerable attention has been given to the elimination of pathogenic microbiota from meat in recent years. A

decade ago a group of international experts contemplated an integrated approach to assess the problem of efficacies of various separate or combined production practices or product treatments. The agreement was that strict adherence to measures of Good Agricultural Practice (GAPs) and Good Manufacturing Practice (GMPs) during production and processing should be the keystone of meat food safety strategies, and that decontamination steps of whatever nature should serve as supplementary means of assuring safety.

The Meat Safety Act (RSA, 2000b) makes provision for the establishment of meat safety schemes which has resulted in many South African abattoirs striving towards the implementation of a Hygiene Management System (HMS) as many deficiencies still exist within abattoirs. Regulatory authorities have sought improvement of the microbiological safety of meat in general by requiring the implementation of hazard analysis critical control point (HACCP) systems in all meat packaging plants. The procedures currently recommended and employed for developing HACCP systems in the meat industry are based on subjective assessments of the microbiological effects of operations in production processes, and of the actions taken to control microbiological contamination. It has therefore been suggested that HACCP systems at meat plants should, amongst others, be based on microbiological data that allow estimation of the numbers of indicator organisms on products at various stages of processing (Gill *et al.*, 2003). It is furthermore generally accepted that the effective implementation of a HACCP system is not feasible without intact prerequisite programmes. Such prerequisite programmes are

focused mainly around curbing undesirable microbiological contamination and growth.

The aims of the study are therefore:

- to assess the survival of, amongst others, staphylococci in fresh vacuum-packed red meat stored at 5°C and 18°C in order to determine which microorganisms will predominate during inappropriate storage practices. The 18°C storage temperature simulates conditions where a breach in the cold cold-chain occurs;
- to report on the occurrence of *Staphylococcus* on red meat, prior to the vacuum-packing process and within the corresponding bioaerosols of the deboning room of a high throughput red meat abattoir, as well as on the relationship between the numbers of *Staphylococcus* bacteria found on the red meat and in aerosols; and
- to assess the presence of staphylococci on the hands of the meat handlers, their aprons and working surfaces used in cutting and trimming of the meat cuts, as well as on the conveyor belt.

Using the data obtained in this study, suggestions will be made as to curbing the presence of staphylococci which would aid in the quality control and assurance of the final product. The recommendations made in this study should assist the specific plant to identify shortcomings pertaining to good manufacturing practices of the meat handlers as well as the cleaning and sanitation practices of the deboning room.

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CHAPTER 2

STAPHYLOCOCCAL PRESENCE IN FRESH VACUUM-PACKED RED MEAT AT DIFFERENT STORAGE TEMPERATURES

❖ This chapter has been submitted for publication to the journal: *Food Control*

2.1 ABSTRACT

It is well known that both the type of packaging and the extrinsic environment affect the quality and shelf life of food. Depending on the amount and composition of the initial microbial load on the product, changes in the storage environment of the food can have a detrimental effect on the product shelf life. In this study, fresh vacuum-packed red meat samples were randomly collected from the deboning room of a high throughput red meat abattoir. The samples were exposed to storage temperatures of 5°C and 18°C respectively. Because of the excessive handling of the product prior to vacuum packing the samples were analysed for staphylococci and total viable counts. The *Staphylococcus* genus was further characterised into species by using biochemical assays. All samples stored at 18°C presented early signs (after 48 hours) of microbial proliferation. As expected, both viable counts and staphylococcal presence were approximately 60% lower at 5°C than at 18°C. The following *Staphylococcus* species were identified: *Staphylococcus aureus; S. epidermidis; S. hominis; S. cohnii cohnii; S. lugdunensis*; *S. intermedius* and *S. xylosus*. All of these species are normally associated with the human skin and oral environment while *S. cohnii cohnii* can be traced to urinary infections. Because of the association of the majority of these species with humans, it was deduced that food handlers need to be educated on the importance of proper, safe and hygienic working practices. The lack of such hygienic practices could lead to higher *Staphylococcus* counts and could result in the formation of toxins within the product. Properly functioning

prerequisite programmess (PRPs) and good manufacturing practices (GMPs) should also contribute to the reduction of organisms in the final product.

Key words: *Staphylococcus*; red meat; shelf life

2.2 INTRODUCTION

Vacuum-packaging makes food more convenient by rendering it safe from microorganisms, biological and chemical changes. The shelf life of fresh meat is primarily influenced by the method and effectiveness of packaging (Skandamis and Nychas, 2002). A study by Ahmed *et al.,* (2003) indicates that chemical sprays as a method for improvement in microbiological quality and shelf life, promoted the inhibitory effect on *Staphylococcus aureus.* Likewise, Sheridan *et al.* (1997) also report that a combination of high CO_2 and low temperature storage (0°C) caused a significant reduction of the total viable counts. In order to meet the growing demand for meat, proper packaging has become an indispensable element of processing as consumers increasingly judge the acceptability of the product on its sensorial quality, especially when purchasing fresh meat (Gill, 1996; Insausti *et al.,* 2001; Skandamis and Nychas, 2002). Different packaging techniques such as modified atmospheres have proven their advantages, especially in the inhibition of undesirable microbial growth in perishable products, as well as in the extension of the products` storage life (Buys *et al.*, 2000). As a result the past few years have seen a considerable increase in the trade in vacuum-packed deboned primal cuts (Gracey, 1986). Meat is nevertheless prone to

contamination from the hands and clothes of the staff of abattoirs, from knives and other equipment, as well as from structural surfaces in deboning rooms and during carcass breaking processes. This has imposed increased demands for an adequate packaging system for such products (Nortjé *et al.*, 1990 and Gill *et al.*, 2001).

Staphylococcus species, however, are to a considerable extent host-adapted with about one-half of the known species solely inhabiting humans (*S. cohnii* subsp. *cohnii,* for example), or humans and animals (*S. aureus).* The largest numbers tend to be found near orifices of the body such as the anterior nares. The two most proliferous sources of food contamination are nasal carriers and individuals who are permitted to handle foods with their hands and arms infected with boils or sores (Jay, 2000). Bacteria are the causative agents of two-thirds of foodborne disease outbreaks, with *Staphylococcus aureus* being one of the leading causes of gastroenteritis resulting from the consumption of contaminated food (Benito *et al.*, 2000; Le Loir *et al.*, 2003). Due to the excessive handling of beef carcasses a high percentage of raw meats become contaminated with staphylococci (Doyle, 1989). Because of the manual processing procedure of deboned meat pathogens such as *S. aureus* may access the meat through personnel and equipment (Reij, 2003).

The aim of this study was to identify the occurrence of staphylococci and total viable counts (TVC) in fresh vacuum-packed red meat stored at 5°C and 18°C in order to determine which *Staphylococcus* species would predominate during inappropriate storage conditions. Information on the predomince of the

various microbiota would furthermore cast light on the level of risk associated with red meat after periods of storage under specific conditions.

2.3 MATERIALS AND METHODS

2.3.1 Sampling protocol

Samples were collected from the deboning room of a high throughput red meat abattoir, located in the Free State Province, South Africa. A 5kg meat sample from the hindquarter of a beef carcass was removed from the conveyor belt in the deboning room, after which it was sub-divided and vacuum-packed into 30 smaller individual units. Sampling was performed during the mid-morning working session at the mentioned abattoir. The samples were placed in a cooler box and transported to the laboratory for analysis without delay. The 0-hour sample was immediately analysed (control) while 14 samples were stored at 5°C and 14 at 18°C. The samples stored at 5 °C were analysed at weekly intervals while those stored at 18°C were analysed at 48-hour intervals. This was done to determine the physical and microbial changes taking place within each individually packed sample and yet preventing an oxygen-shock occurring during the breaking of the vacuum if a single sample was to be analysed and re-vacuum packed during each sampling period.

2.3.2 Microbiological analysis

A 10 gram sample was macerated in 90 ml peptone water (Biolab-RSA) by means of a Stomacher (Steward 400) for 3 minutes similar to Mosupye and

Von Holy (1999); Nortjé *et al.*, 1999; Kubheka *et al.* (2001); Fang *et al.* (2002). Serial dilutions were aseptically prepared and 0,1 ml aliquots plated onto media using the spread-plate technique (Lee and Yoon, 2001).

The total viable count (TVC) was estimated using Plate Count Agar, (MERCK, RSA), incubated at 25°C for 48 hours and expressed as LOG CFU.g^{-1} (Bryan *et al.*, 1996). For *Staphylococcus* growth Baird-Parker Agar (Biolab) with 50 ml egg-yolk tellurite emulsion (Merck, RSA) was used and the plates incubated at 35°C for 48 hours (Fang *et al.*, 2002). Black colonies with white margins surrounded by clear zones were regarded as *Staphylococcus aureus*. The colonies were confirmed using the rapid latex agglutination test (Slidex Staph Plus Test Kit, Bio Mérieux). Similar to Nagase *et al.* (2001) the API-Staph system (OMNIMED, RSA) for *Staphylococcus* species identification was used to identify the remaining species. *S. aureus* was used as positive control and *S. epidermidis* as negative control. All analyses were done in triplicate.

2.4 RESULTS AND DISCUSSION

The total viable counts (TVC) in vacuum-packed red meat stored at 5°C are presented in Figure 2.1(A). The initial TVC at 0 hours was 1.3 x 10^4 CFU.g^{-1} but increased to 7.1 x 10^5 CFU.g^{-1} by week 6. The total viable counts observed in this study are contrary to the findings of Sheridan *et al.* (1997) where a significant reduction of total viable counts were observed under high CO_2 and low temperature. This could be due to the fact that some bacteria

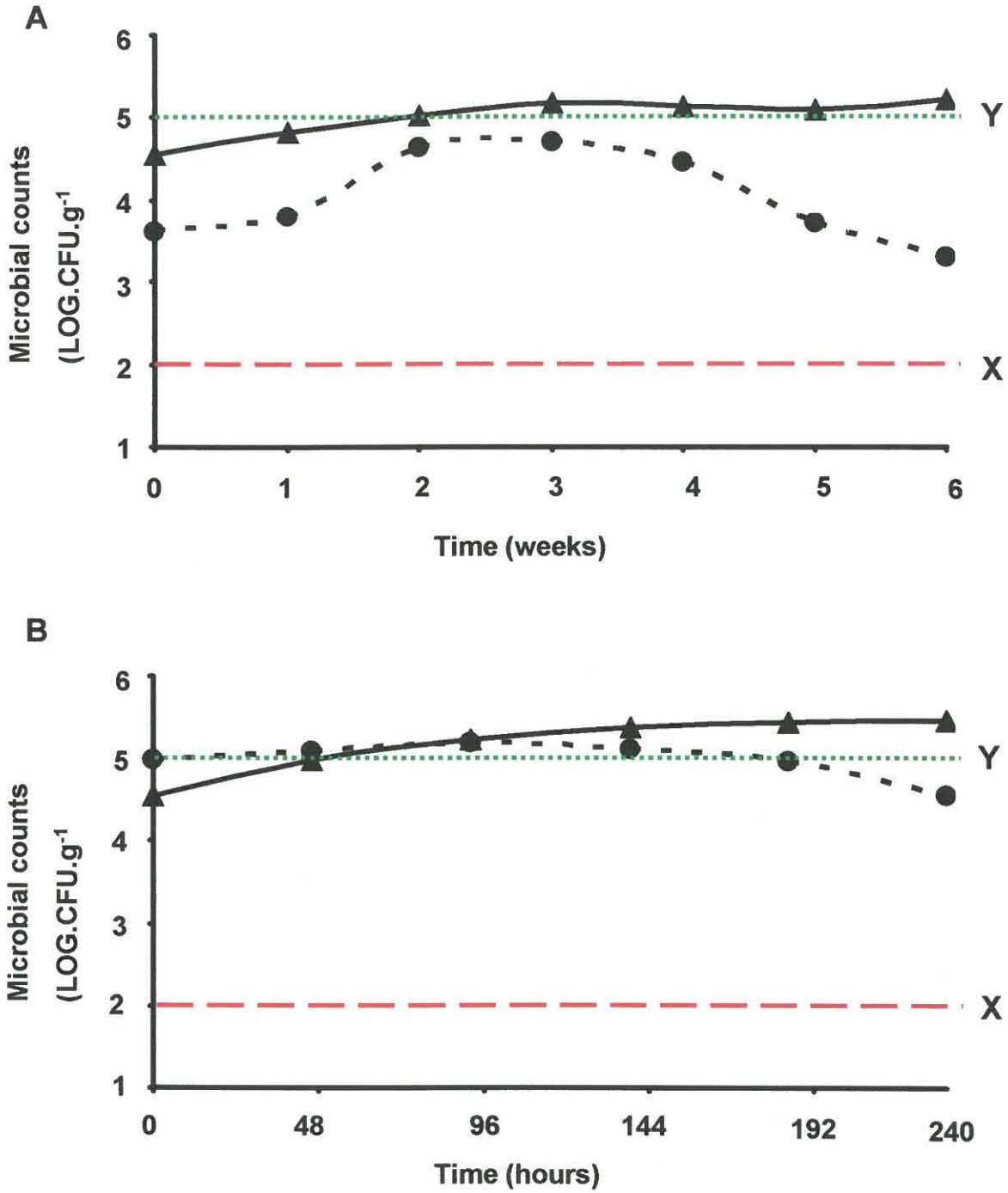

Figure 2.1 The total viable counts —▲— and staphylococci ··●·· presence on fresh vacuum-packed red meat during storage at 5°C (A) and 18 °C (B) respectively. X - National Guideline; Y- Infective Dose Level

are poor competitors and grow slow in the presence of other bacteria that may be present on meat (Cliver, 1990; Doyle, 2002; Karaboz and Dincer, 2002). The presence of staphylococci at 0 hours was 1.4 x 10^3 CFU.g^{-1} and the highest viability of 5.2 x 10^4 CFU.g^{-1} was noted during week 3. Weeks 4, 5 and 6 also indicated the presence of staphylococci. A definite decrease in staphylococcal counts was observed during weeks 5 and 6. This could be related to the competitive nature of other organisms (TVC) due to production of organic acids and gases as well as oxygen within the vacuum-packed meat (Cliver, 1990; Ware *et al.*, 1999).

Despite the fact that the initial total viable counts and staphylococci were relatively high the product showed no visible signs of deterioration or off-odours over the six-week period of analysis at 5°C. The results showed, however, that although the meat product was kept at 5°C and appeared to be sensorially intact, TVC and staphylococci still proliferated. The occurrence of staphylococci on the vacuum-packed meat was expected due to the organism's widespread and ubiquitous nature (Desmarchelier *et al.*, 1999). These bacteria are present on the skin and hair of warm-blooded animals and up to 30-80% of the human population habour enterotoxigenic strains (Atanassova *et al.*, 2001; Nagase *et al*, 2002; Le Loir *et al.*, 2003). The manual handling of the meat prior to vacuum-packing, and especially the deboning process, could explain the bounteous presence of these organisms within the vacuum-packed meat (Minor and Marth, 1976; Atanassova *et al.*, 2001).

Figure 2.1(B) shows the TVC and staphylococcal counts observed in the vacuum-packed red meat stored at 18°C, analysed over 48-hour intervals. The 0 hour TVC was 1.3 x 10^4 CFU.g^{-1} and within 48 hours a definite increase occurred, to 4.8 x 10^5 CFU.g^{-1}. The TVC counts increased further after every 48-hour interval with the highest level noted after 240 hours at 2.4 x 10^7 CFU.g^1. A study done by Nortjé *et al.* (1999) suggests that total viable counts in the region of 10^7 g^{-1} in meat is regarded as the upper limit of acceptability. Staphylococci had an initial 0 hour count of 1.4 x 10^3 CFU.g^{-1} after which a definite increase in counts was observed. The highest staphylococcal presence was observed after 144 hours with counts *circa* 1.3 x 10^5 CFU.g^{-1}. A decrease in staphylococci was, however, observed at 192 hours of storage at 18°C. Staphylococci reached levels of 3.5 x 10^4 CFU.g^{-1} after 240 hours at 18°C. The staphylococcal counts exceeded by far the national guideline of 100 CFU.g^{-1} as well as the infective dose as stipulated by the Department of Health (RSA, 2000). The high counts of staphylococci obtained during this study are of particular concern as this points to contamination of the product by the meat handlers when processing the meat prior to vacuum-packaging (Steele and Stile, 1981).

Notable sensorial changes of the vacuum-packed meat were observed after 48 hours of exposure to temperatures of 18°C. The meat had a definite change in colour; formation of a slimy layer on the surface of the meat and an off-odour were noted. By 96 hours of exposure all vacuum-packed meat samples were bloated, indicating the formation of gas within the packages. There was also a change in texture and a very strong off-odour. The off-odour

Table 2.1 Staphylococci species identified on red meat at 5°C and 18°C respectively.

***Staphylococcus* species**	**Temperature**			
	5°C	**%**	**18°C**	**%**
Staphylococcus aureus	8	**50**	15	**50**
S. intermedius	3	**19**	7	**23**
S. xylosus	2	**12**	3	**10**
S. epidermidis	2	**12**	2	**7**
S. cohnii cohnii	1	**6**	1	**3**
S. lugdunensis	0	**0**	1	**3**
S. hominis	0	**0**	1	**3**
	16	**100**	30	**100**

production can be indirectly related to the temperature at which the vacuum-packed meat was stored (Gill, 1996; Sheridan *et al.*, 1997).

Table 2.1 shows the *Staphylococcus* species identified on the vacuum-packed red meat at 5°C and 18°C respectively. *Staphylococcus aureus* was identified on 50% of the samples at both temperatures (15 identified at 18°C and 8 at 5°C). This was to be expected as the skin of humans is reported to be the most common source of *S. aureus* and from this point the organism finds its way into the air and clothes from which it may further contaminate foods (Jay, 2000). The second most abundant species was *S. intermedius*, with 7 (23%) identified at 18°C and 3 (19%) at 5°C and this species is a known pathogen occasionally associated with human infection, especially in superficial infections of the ear canal (Tanner *et al.*, 2000). The presence of *S. xylosus* was noted on 10% of the samples at 18°C and 12% at 5°C. This organism is associated with nasal dermatitis which is commonly known as "sore nose", and is sometimes temporally related to stress and trauma. It has previously been isolated from the teat skin of cows as well as from the nares of humans (Nagase *et al.*, 2002). The presence of this organism thus not only points to human contamination but also to possible slaughter contamination. *Staphylococcus epidermidis* represented 7% of the species in the samples evaluated at 18°C and 12% at 5°C while *S. cohnii* represented 3% at 18°C and 6% at 5°C. *Staphylococcus cohnii is* a sub-species of *S. saprophyticus.* This organism is the second most frequently encountered agent of acute urinary tract infections and is often isolated from the urine of young, sexually active females with symptoms of acute urinary tract infections (UTI). It has

also been shown to be responsible for urinary infections in small animals (Martineau *et al.*, 2000; Songer, 2004). *Staphylococcus lugdunensis* and *S. hominis* are sub-species of *S. epidermidis* both were present at 3% at 18°C and no growth could be detected at 5°C for either of the species. Jay (2000) and Le Loir *et al.* (2003) report that the majority of the above-mentioned species are of potential interest in foods as several staphylococcal species other than *S. aureus* reportedly produce staphylococcal enterotoxins (SEs). Some examples among the coagulase negative species are *S. cohnii, S. epidemidis, S. xylosus* and *S. haemolyticus*; however, most of the above are not tested for in routine evaluation of staphylococcal food poisoning.

The viability of staphylococci and total viable counts could be dictated by the high initial presence of these organisms within the product at the time of vacuum-packing, as observed in the 0 hour counts of both TVC and staphylococci. The initial presence of these bacteria in such high counts is likely to be a result of the high level of manual handling of the product during stages such as deboning (Atanassova *et al.*, 2001). Desmarchelier *et al.* (1999) emphasises that the hands of food handlers are an important secondary source of contamination of staphylococci, especially during meat processing. Staphylococci counts higher than the infective dose level of 10^5 CFU.g^{-1} where observed at 18°C, suggesting possible toxin production which might lead to food poisoning (RSA, 2000), not only by *Staphylococcus aureus* but also by other species such as *S. hyicus* and *S. intermedius*. Both of these species are coagulase positive and may produce staphylococcal enterotoxins (Desmarchelier *et al*, 1999). Although it must be noted that keeping of meat at

18°C for prolonged periods by producers is unlikely, it is important to note that even at 5°C the staphylococci counts were only marginally below the infective limit of 10^5 CFU.g^{-1} (RSA, 2000).

The high levels of staphylococci could suggest insufficient hygiene and working practices of the meat handlers during the processing stage, as well as a lack of sterilization of utensils and working surfaces. It is furthermore suggested that the management of the abattoir revise the hygiene management system as this would aid in the identification of poor manufacturing practices by the meat handlers. Moreover it is important that the workers report all illnesses no matter how minor they may seem, such as ear infections caused by *Staphylococcus intermedius,* to the resident medical officer or nurse as various staphylococcal species are known enterotoxin producers (Jay, 2000). It would be advisable that the abattoir in this study consider the various types and techniques of vacuum-packaging especially those using modified atmospheres. Finally it is advised that the high throughput abattoir review the present illness-reporting programme and that health checks on meat workers within the deboning room be performed on a regular basis. The quality control system in use at the abattoir should also be reviewed.

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CHAPTER 3

THE ETIOLOGY OF *STAPHYLOCOCCUS* SPECIES ASSOCIATED WITH THE DEBONING ROOM OF A HIGH THROUGHPUT ABATTOIR

❖ This chapter has been submitted for publication to the journal: *Food Microbiology*.

3.1 ABSTRACT

Meat is a highly perishable product and the microbiological safety thereof has become an important element to both the consumer and industry. Therefore its safety depends on the application of effective control measures at all stages of the production chain, literally from farm to fork. In this study, meat and air samples were collected randomly from a deboning room of a Grade A (high throughput) abattoir, over a period of 4 weeks. The samples were analysed for total viable counts as well as for the presence of *Staphylococcus* species. Total viable counts in the meat ranged between 2.9 x 10^2 and 3 x 10^3 CFU.g^{-1} while the staphylococci levels were well below the proposed maximum limit of 100 CFU.g^{-1} as required by the Department of Health (RSA, 2000). The surrounding atmospheric environment of the deboning room was sampled for the mentioned microbiota using a Microbial Air Sampler (SAS, Super 90). The total viable counts measured in bioaerosols recorded ranged between 14 and 30 CFU.m^{-3}. The bioaerosols enumerated in the deboning room tested positive for the presence of a number of *Staphylococcus* species with the highest level of 10 CFU.m^{-3}. The mean airborne *Staphylococcus* counts were approximately 38% compared to the *Staphylococcus* counts in the meat while the TVC in the air were 18% compared to the meat. The counts obtained for *Staphylococcus* point to contamination of the product by meat handlers during processing of the meat.

Key words: *Staphylococcus*; red meat; abattoir deboning room

3.2 INTRODUCTION

In the meat industry there clearly are several pathways through which pathogenic and spoilage organisms can be introduced to the final product. One potential source that is often overlooked is air contamination of meat that can occur during storage and processing (Cundith *et al.*, 2002). Ren and Frank (1991) report that the microbiota on bioaerosols could originate from plant workers, floor drains, the ventilation system, openings between rooms and by conveyor systems, and that very recently high water sprays have been implicated as a major source of bioaerosols. It has furthermore been found that airborne microbes originate from undetected contamination on surfaces that are insufficiently cleaned during the normal cleaning and sanitation procedures of processing plants.

Inner tissue of healthy bovine carcasses has been reported to contain few or no microorganisms (Frazier and Westhoff, 1988; Nortjé *et al.*, 1990 and McEvoy *et al.*, 2000). However, when animals are slaughtered, bacteria from the hide, the gut and the processing environment may contaminate the surfaces of the meat. According to Gill *et al.* (2000) wide variations exist between processes and the extent to which carcasses are contaminated. Lack of proper processes and personal hygiene at any stage in the processing, storage, transport, handling, cooking and serving of food products, including meat products, can result in contamination and cross-contamination (Bell, 1997). Activities in the deboning room may further

contaminate meat especially during cutting and trimming, from the knives and saws used, the conveyor belts, tables, air, water and finally the meat handlers (Frazier *et al.*, 1988). It is during these handling and processing stages that meat becomes contaminated with different pathogenic organisms such as staphylococci and other foodborne pathogens (Gordon-Davis, 1998 and Snyder, 2003). In many parts of the world, *Salmonella* species, particularly *Salmonella derby* and *Staphylococcus aureus,* are reported to be frequent contaminants on fresh meat (Ockerman *et. al.*, 2001).

The presence of *Staphylococcus aureus* in products for human consumption is important to the food industry, as some strains are the cause of foodborne intoxication (Desmarchelier *et al.*, 1999). Because of the ubiquitous nature of staphylococci these organisms are commonly found on the skin, hair and mucosal membranes of mammals and birds, as well as in the general environment (Vanderlinde *et al.*, 1999; Le Loir *et al.*, 2003). Several *Staphylococcus* species have been found to be part of the oral microbiota of humans (Sherertz *et al.*, 2001; Smith *et al.* 2001). Nagase *et al.,* (2001) notes the following staphylococcal species as predominant in humans: *S. epidermidis*; *S. warneri*; *S. hominis*; *S. capitis* and *S. aureus,* with the following predominant in pigs, cattle and poultry: *S. xylosus*; *S. aureus*; *S. sciuri*; *S. cohnii subsp. cohnii*; *S. saprophyticus* and *S. epidermidis*.

The presence of staphylococci is therefore usually indicative of contamination from the skin, mouth or nose of food handlers, or from inadequately cleaned equipment or raw animal products. It is therefore the purpose of this study to

investigate on the occurrence of *Staphylococcus* species on meat prior to the vacuum-packaging process in the deboning room of a high throughput red meat abattoir, as well as the corresponding bioaerosols present in this environment. The purpose was furthermore to investigate the relationship between the numbers of *Staphylococcus* and total viable counts found on the red meat and in the bioaerosols since fresh meat is a rich medium for microbial growth.

3.3 MATERIALS AND METHODS

3.3.1 Collection of samples

Meat samples were collected on a weekly basis over a one-month period from the deboning room of a high throughput red meat abattoir. In South Africa a high throughput abattoir is classified as a Grade A abattoir, handling more than one hundred slaughter units per day. The samples were collected randomly during the morning shift at half-hour intervals over a 5-hour period. The samples were collected from the conveyor belt prior to the vacuum-packing process and stored in sterile Whirl-Pak bags (Nasco, USA). Finally the samples were placed in a cooler bag and transported to the laboratory for analysis without delay.

3.3.2 Microbiological analysis

The samples were blended (Waring Blender, USA), and 10 grams of the macerated sample aseptically weighed off and transferred to 90 ml peptone water (Biolab-RSA), and homogenised in a Seward Stomacher 400 for 3

minutes (Nortjé *et al.*, 1999; Fang *et al.*, 2002). Serial dilutions were aseptically prepared and 0,1 ml aliquots plated onto media using the spread-plate technique.

The total viable count (TVC) was using Plate Count Agar (MERCK, RSA). The plates were incubated at 25°C for 48 hours, enumerated and expressed as LOG CFU.g^{-1} (Bryan *et al.*, 1996). For *Staphylococcus* growth Baird-Parker Agar (Biolab, RSA) with 50 ml egg-yolk tellurite emulsion (Merck, RSA) was used and the plates incubated at 35°C for 48 hours (Fang *et al.*, 2002). *Staphylococcus aureus* colonies were black with white margins surrounded by clear zones. The colonies were confirmed using the rapid latex agglutination test (Slidex Staph Plus Test Kit, Bio Mérieux). Similar to Nagase *et al.* (2001) the API-Staph system (OMNIMED, RSA) was used to identify the remaining *Staphylococcus* species. All analyses were done in triplicate.

3.3.3 Bioaerosol samples

Bioaerosol samples were collected using the Microbial Air Sampler (SAS-Super 90). This apparatus measures aerosolised bacteria by means of impaction on agar as described by Theron (2003). The air sampler was pre-calibrated at 28.3 l/min prior to sampling and all the removable components of the air sampler were pre-autoclaved and disinfected with 70% ethanol between each sampling run. Impaction on 55 mm Rodac plates containing solidified Baird Parker and Plate Count Agar was used for the islolation of the mentioned microbiota. Bioaerosol sampling was performed 1.5 m above the floor and in the centre of the working and breathing zone of workers, after

which sampling plates were transported to the laboratory on ice and incubated at 35°C staphylococcal growth and 25°C for total viable counts for 48 hours. The positive-hole method was applied for corrections of microbial coincidence (Macher, 1989). The Slidex Staph Plus Test Kit, (Bio Mérieux) and the Analytical Profile System (API-Staph; OMNIMED RSA) were used for identification of *Staphylococcus* species.

3.4 RESULTS AND DISCUSSION

High total viable counts were recovered from all the meat samples during the 4-week sampling interval with counts ranging between 2.9×10^2 and 3×10^2 $CFU.g^{-1}$ as shown in Figure 3.1(A). Relatively low *Staphylococcus* counts were recorded in week 1 compared to the counts obtained during the shelf life study (Chapter 2), while week 2 depicted higher counts of 2.3×10^1 $CFU.g^{-1}$. South African legislation (RSA. Department of Health, 1999) also emphasises the importance of the frequency of hand washing of food handlers, as hands are rarely free from microorganisms, especially staphylococci (Nel, 2003). It is important to note that carcasses from feedlot cattle also showed increased numbers of staphylococci (week 2) compared to the other three weeks during which only non-feedlot cattle where slaughtered. Bacon *et al.* (2001) reports that under feedlot conditions animal stress increases, contributing to the spreading of bacteria both internally and externally. This could explain the higher staphylococcal counts obtained. The levels for *Staphylococcus* on meat were well below the proposed maximum limit of 100 $CFU.g^{-1}$ as required by the Department of Health (RSA, 2000). A previous study done by Nel

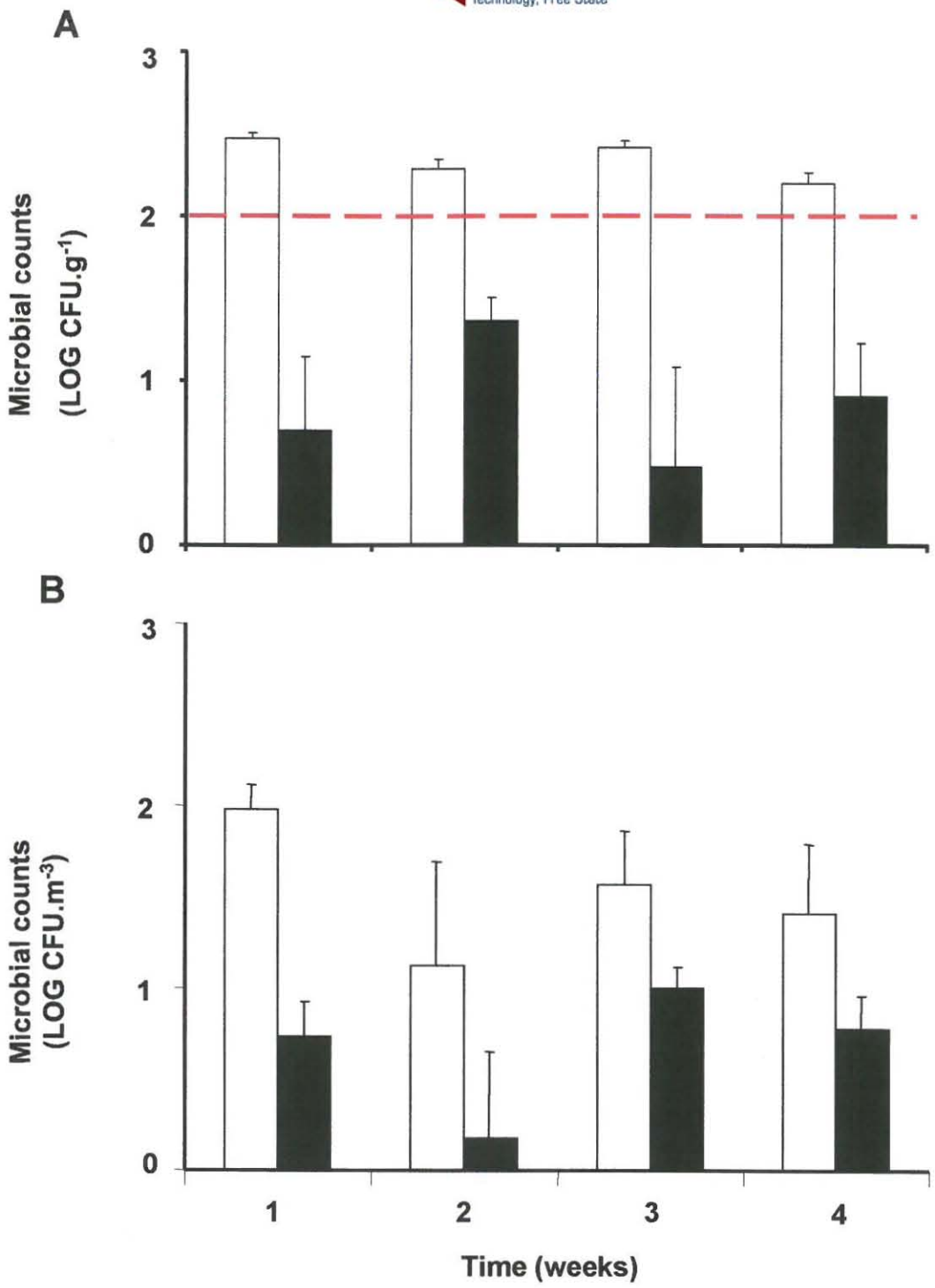

Figure 3.1 The occurrence of *Staphylococcus* ■ and □ total viable counts on red meat (A) and total viable counts measured in bioaerosols and airborne staphylococci (B) within the deboning room of a high throughput abattoir. X - National Guideline for *S. aureus*

(2003) showed *Staphylococcus* levels ranging between 3.8 x 10^3 to 2.4 x 10^5 CFU.g^{-1}, suggesting a possible food poisoning hazard. In this study the staphylococci levels were, however, also below the infective dose level of 10^{-5} CFU.g^{-1} thus suggesting no immediate danger of toxin production (Forsythe, 2000; Atanassova *et al.*, 2001).

The highest airborne total viable counts (TVC) were recorded during week 1 with levels of 95 CFU.m^{-3} as shown in Figure 3.1 (B). During the remaining three weeks lower total viable counts were observed with levels between 12 and 30 CFU.m^{-3}. Higher counts of airborne staphylococci were observed during week 3 (10 CFU.m^{-3}), while week 2 showed a decrease in counts to as low as 1.5 CFU.m^{-3}. The decrease in the number of bioaerosols could be attributed to the improved ventilation system implemented at the high throughput red meat abattoir during our sampling period. The Department of Health (RSA, 2000) proposes a maximum limit of 100 CFU.g^{-1} for *Staphylococcus aureus* in meat but no maximum limit pertaining to bioaerosols.

The results from the study into the species distribution of staphylococci are shown in Table 3.1. The following species were isolated: *Staphylococcus aureus; S. epidermidis; S. capitis; S. auricularis; S. homonis; S. saprophyticus; S. haemolyticus S. simulans* and *S. sciuri. Staphylococcus aureus* was dominant in both meat and air samples and represented 27% of the staphylococcal population. This was expected since *S. aureus* is a principle contaminant on the skin of humans and animals thus explaining the

Table 3.1 The general occurrence of *Staphylococcus* species identified on the red meat and in the bioaerosol environment of the deboning room

***Staphylococcus* species**	**Deboning Environment**				
	Meat[a]	**%**	**Bioaerosols[b]**	**%**	**Total % (a + b)**
Staphylococcus aureus	15	30	12	24	**27**
S. epidermidis	10	20	10	20	**20**
S. capitis	2	4	6	12	**8**
S. auricularis	6	12	4	8	**10**
S. hominis	4	8	5	10	**9**
S. saprophyticus	6	12	5	10	**11**
S. haemolyticus	3	6	6	12	**9**
S. simulans	2	4	2	4	**4**
S. sciuri	2	4	0	0	**2**
	50	100	50	100	**100**

(a + b)% = the total percentage of the isolated species in both the meat and bioaerosols

occurrence of this organism in both raw meat and the air environment of the deboning room (Frazier and Westhoff, 1988; Adams and Moss, 1997; Chambers, 2001). Furthermore von Eiff *et al.*, (2001a, b) also mention the fact that these bacteria are abundant in the nasal passages of many humans.

The second most prominent organism was *Staphylococcus epidermidis* (20%) and according to Miragaia *et al.* (2002) and Otto *et al.* (2000) *S. epidermidis*, sub-species *S. capitis*, is one of the staphylococcal species most frequently isolated amongst the microbiota of humans. These authors also point out that the organism has emerged as a major pathogen in nosocomial infections while *S. hominis* (9%) isolated, *S. capitis* (8%), *S. haemolyticus* (9%) and *S. simulans* (4%) have all been implicated in various infections. Furthermore Smith *et al.* (2001) report *S. epidermidis* and *S. aureus* to be the most frequently inhabiting oral microbiota with *S.haemolyticus, S. homonis, S capitis, S. saprophyticus* and *S. simulans* being less prevalent. *Staphylococcus saprophyticus* (11%) is associated with urinary tract infections, especially amongst young sexually active women (Latham *et al.*, 1983; Fihn *et al.*, 1998; von Eiff *et al.*, 2001a). Some of the staphylococci such as *S. sciuri* stem primarily from rodents and low counts of *S. sciuri* (2%) could possibly be related to contamination from rodents in the abattoir (Schwarz *et al.*, 2000; Shang *et al.*, 2001).

The total viable counts enumerated from the meat were well below 10^{-7} $CFU.g^{-1}$ which is regarded as the upper limit of acceptability as stated by Department of Health (RSA, 2000). Many of the above-mentioned species

were isolated both in the air and in the meat samples during the present study. It is important to note that the meat handlers were not wearing any mouth or beard masks and that at the time of sampling workers were singing while performing their work. This could partially explain the species isolated within the aerosol environment of the deboning room.

Brown *et al.* (2000) generally acknowledges the fact that traditional meat inspection procedures cannot guarantee that consumers will not be exposed to infectious doses of meat-borne pathogens and that the hands of food handlers generally reflect their environment as well as the habits of the individual. The guidelines regarding the reporting of illnesses by meat handlers should be closely monitored and revised so as to rectify the present practice according to which only influenza, colds and diarrhoea are reported. Reporting of urinary tract infections should be deemed equally as important as the other illnesses. Although the counts of staphylococci identified in this study were below the national guideline it would be advisable that the meat handlers within the deboning room be screened on a regular basis, since the presence of these organisms could act as indicators of their personal hygiene and health status.

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CHAPTER 4

THE PREVALENCE OF STAPHYLOCOCCI ON SURFACES IN THE DEBONING ROOM OF A HIGH THROUGHPUT RED MEAT ABATTOIR

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4.1 ABSTRACT

Apart from humans and animals, contaminated surfaces are one of the major sources of contamination in red meat abattoirs. Even after cleaning and disinfection of surfaces certain species of microbiota remain on the surfaces because of either the composition of the surface, or the ability of the organism to adhere thereto. It has been reported that *Staphylococcus* organisms form biofilms on surfaces and continuously re-contaminate the product during processing. In this study samples were collected from various surfaces in the deboning room of a high throughput abattoir and investigated for total viable counts as well as total staphylococci counts. The total viable counts (TVC) from workers" hands and working surfaces were relatively high and well above the national guideline of 100 CFU.cm^2 for working surfaces. The mean staphylococci counts from the surfaces were 19 CFU.cm^2. The following staphylococcal species were identified: *Staphylococcus aureus; S. epidermidis, S. warnei; S. lugdunensis; S. xylosus; S. haemolyticus; S. hominis; S. sciuri; S. saprophyticus; S. capitis; S. simulans* and *S. auricularis*. The presence of the above staphylococci pointed to inadequate cleaning and sanitation practices resulting in definite contamination of the meat by surfaces within the deboning room.

Key words: *Staphylococcus*, abattoirs, surfaces

4.2 INTRODUCTION

Meat inspection authorities are increasingly seeking to control the contamination of raw meat by pathogenic bacteria through mandating the implementation of Hazard Analysis Critical Control Point (HACCP) systems at meat processing and packing plants. Many abattoirs in South Africa, however, are still in the process of implementing good manufacturing practices (GMPs) as a prerequisite programme to HACCP. This addresses not only the techniques used by the slaughtermen and other personnel, but also the construction of the building and the design of the equipment which will facilitate the separation of clean and dirty procedures, as well as abattoir cleaning and disinfection procedures (Reuter, 2000). The need to maintain food contact surfaces in a hygienic state is of obvious importance.

The contribution of surface contamination from walls, tables, floors and equipment used during food processing to product shelf life and quality has been the subject of much debate. It is generally accepted that microbial loads on surfaces and equipment vary in different food plants, depending on the microbial quality of the food and the cleaning programmes in operation (Evans *et al.*, 2004). The red meat industry has adopted technologies such as hot water or steam vacuuming, steam pasteurization, ambient temperature or hot water spray washing with or without organic acid solution rinsing, to reduce biofilms on surfaces. In spite of this, microbial contamination from surfaces remains a major concern within the industry (Bacon *et al.*, 2002). Nortjé *et al.* (1990) report that many opportunities still exist for contamination

of the meat from the hands and clothes of the staff, from knives and other equipment, and from structural surfaces in deboning rooms and retail premises that harbour undesirable microbiota.

Many studies have shown that when animals are slaughtered, microbiota remain on the surface of the carcass and despite the decontamination processes carried out, some of the microbiota survive. Consequently, employees and improperly sanitized contact surfaces such as belts, tables, saw blades and cutting boards serve as potential sources of contamination through the formation of biofilms (Nortjé *et al.*, 1990). Staphylococci are part of the microbiota most commonly found in the meat industry and these bacteria serve as indicators of excessive human handling (Midelet and Carpentier, 2002; RSA, 2000).

The aim of this study was to assess the presence of staphylococci on the hands of meat handlers, their aprons, working surfaces used for cutting and trimming the meat cuts, as well as on the conveyor belt that transports the meat to the vacuum-packaging room. Finally, the various *Staphylococcus* species and total viable counts isolated from the mentioned surfaces within the deboning room were identified in order to cast light on the distribution and possible origin of the various staphylococcal contaminants.

4.3 MATERIALS AND METHODS

4.3.1 Surface sampling protocol

Surface samples were collected during the deboning process of a high throughput abattoir located in the Free State Province, South Africa. During deboning, meat is removed from the skeletal bones of the hanging carcass and then cut into various retail cuts, where it is placed on stainless steel tables and then manually trimmed to remove excess fat. The meat is then placed onto the conveyor belt that transports it to a separate room where it is vacuum-packed. One hundred surface samples were collected from these various localities using 55mm surface contact plates (Rodac Nunc, Denmark). The recovery of total viable counts (TVC) and staphylococci from stainless steel tables, conveyor belts, aprons, hand prints from the meat handlers and the steel mesh gloves worn on the left hand were sampled.

4.3.2 Microbial quantification

The total viable count (TVC) was quantified using Plate Count Agar, incubated at 25°C for 48 hours (Bryan *et al.*, 1996). For *Staphylococcus* sp. Baird-Parker Agar (Biolab) with 50 ml egg-yolk tellurite emulsion (Merck, RSA) was used and the plates incubated at 35°C for 48 hours (Fang *et al.*, 2002). Black colonies with white margins surrounded by clear zones were regarded as *Staphylococcus aureus*. The colonies were confirmed using the rapid latex agglutination test (Slidex Staph Plus Test Kit, Bio Mérieux). Similar to the study undertaken by Nagase *et al.* (2001) the API-Staph system

(OMNIMED, RSA) was used to identify the remaining *Staphylococcus* species. All analyses were done in triplicate.

4.4 RESULTS AND DISCUSSION

With regard to the average total viable counts (TVC), these counts were $>10^4$ CFU.cm^{-2}. The relatively high numbers of total viable counts were expected because of bacteria being transferred on either gloves and equipment or both, from the more to the less contaminated sites. The contamination could furthermore be deposited on the meat afresh from sources such as improperly cleaned equipment that cannot be disassembled for cleaning purposes, thus resulting in hard-to-reach sections being irregularly and inadequately cleaned (Gill and Jones, 1999). The South African Department of Health (RSA, 2000) proposes a maximum limit of 100 CFU.g^{-1} for *Staphylococcus aureus* in meat with 10^5 CFU.g^{-1} being indicative for food poisoning. There are no guidelines for surfaces contaminated by *Staphylococcus* species. There are however guidelines prescribing the total microbial contamination allowed on surfaces, being 100 CFU per surface (cm^{-2}).

In Table 4.1 the numbers of staphylococci on the various surfaces within the deboning room is indicated. The levels of staphylococci ranged between 12 and 28 CFU.cm^{-2} with stainless steel tables showing the highest numbers of 28 CFU.cm^{-2}. Kusumaningrum *et al.* (2003) suggest that contamination of bacteria on stainless steel surfaces with levels of 100 CFU.cm^{-2} can be

Table 4.1 The average staphylococcal growth on surfaces within the deboning room of a high throughput abattoir

Surfaces	Staphylococci (CFU.cm^{-2})	Total Viable Counts
Tables	28	> 10^4
Conveyor belt	22	> 10^4
Aprons	17	> 10^4
Left Hand Glove		
Thumb	21	> 10^4
Forefinger	23	> 10^4
Left Hand No Glove		
Thumb	18	> 10^4
Forefinger	15	> 10^4
Right Hand No Glove		
Thumb	12	> 10^4
Forefinger	13	>10^4

regarded as low. When compared with the levels obtained in this study, the latter would be regarded as moderately contaminated at levels of < 10 CFU.cm^{-2}. The higher staphylococcal levels on the steel mesh gloves (23 CFU.cm^{-2}) could be related to the build-up of bacteria as the work progressed through the day or to the effects of fat and tissue debris contaminating the hands and the steel mesh gloves (Legg *et al.*, 1999). Another reason for the higher counts on gloves could be that *Staphylococcus* accumulates as hands perspire and may multiply on hands that are encased in gloves for long periods. This could also explain why the left-hand prints had more staphylococci than the right hand prints because for the majority of right-handed workers only the left hand is encased in the steel mesh glove during the work session. Due to perspiration the hand is inclined to have more staphylococci on the surface of the skin. The staphylococci on the aprons could most likely be related to the transfer of the microbes during the processing of the various retail cuts from the carcass to the working surfaces as well as the fact that the food handlers tend to wipe their hands on their aprons. Because of the one-way conveyor belt system, the retails cuts were also prone to recontamination from the various meat handlers with the placement of these cuts onto the conveyor belt.

Due to the clump-like structure of *Staphylococcus* some cells have been found to adhere to stainless steel surfaces. Scanning electron micrographs demonstrated that some cells were found in crevices on stainless steel surfaces. The presence of these *Staphylococcus* species on the various surfaces may thus be due to contact with contaminated objects or indirectly

through airborne particles after which the organism latched onto the surface. Certain bacteria attach to surfaces as their predominant form of survival in nature and in man-made ecosystems (Scanga *et al.*, 2000; Kusumaningrum *et al.*, 2003).

In Table 4.2 the occurrence of *Staphylococcus* species across the various surfaces is shown. *Staphylococcus aureus* represented 24% of the species while 70% of these organisms were noted on the steel mesh gloves and hands of the meat handlers in particular. This was expected due to the carcass processing procedures within the deboning room, as the sources of contamination were likely to be the meat handlers working in the processing plant (Schlegelová *et al.*, 2004). The second most abundant species was *S. epidermidis* 16% of this species was isolated from the table, conveyor belt, aprons and fingerprints of meat handlers. *Staphylococcus sciuri*, *S. saprophyticus* and *S. capitis* were noted on 9% of the surfaces sampled.

Staphylococcus warneri and *S. simulans* were isolated from 7% of the surfaces and *S. hominis* from 5%. *S. lugdunensis*, *S. xylosus*, *S. haemolyticus* and *S. auricularis* represented the remainder of the species isolated from the surfaces. Of the above species identified, *S. aureus* and *S. epidermidis* have been isolated from humans as commensals (nares and skin) and also have the greatest pathogenic potential, while *S. saprophyticus* is known as a common cause of urinary tract infections, especially in females (Foster, 2002).

Table 4.2 The occurrence of *Staphylococcus* species identified on various surfaces within the deboning room

***Staphylococcus* species**	**Apron**	**Table**	**Conveyor belt**	**Left hand steel mesh glove[1]**		**Left hand no glove**		**Right hand no glove**		**Percentage occurrence %[2]**
				T	**F**	**T**	**F**	**T**	**F**	
S. aureus	2	1	1	1	2	3	1	1	1	**24**
S. epidermidis	3	2	1					3		**16**
S. warneri	1		1			2				**7**
S. lugdunensis	1		1							**4**
S. xylosus			1						1	**4**
S. haemolyticus						1			1	**4**
S. hominis		2						1		**5**
S. sciuri	1						2		2	**9**
S. saprophyticus		1	2				2			**9**
S. capitis		1		2	2					**9**
S. simulans		1		2	1					**7**
S. auricularis									1	**2**
Isolates n = 55	**8**	**8**	**7**	**5**	**5**	**6**	**5**	**5**	**6**	**100**

1: T = Thumb; F=, Forefinger

2: Total species identified / 55 (isolates) x 100 = %

Kawamura *et al.* (1998) and Smith *et al.* (2001) report the following species of staphylococci to be from the carriage of humans and part of the human biota: *Staphylococcus epidermidis, S. aureus, S. haemolyticus, S. hominis, S. warneri, S. capitis, S. saprophyticus, S. xylosus, S. warneri, S. lugdunensis, S. cohnii, S. auricularis* and *S. simulans.* These species reported by the authors mentioned-above represent 92% of the organisms isolated from the surfaces in this study and their presence could be related, amongst other things, to the fact that none of the meat handlers within the deboning room were wearing facial masks during the time of sampling. The fact that the majority of workers used their hands to wipe away irritations from their mouths and noses during processing of the meat was furthermore disturbing. Handling operations of the product within the deboning room should be regarded as critical, and ignorance in this regard may suggest that the workers were not informed about the importance of wearing facial masks (Martínez-Tomé *et al.*, 2000; Den Aantrekker *et al.*, 2003).

The risk of foodborne infection associated with cross-contamination depends on the level of contamination on the surfaces and its probability of being transferred to the foods to be consumed. In this study *Staphylococcus aureus* has been proven to remain viable on stainless steel surfaces for hours or even days after contamination (Kusumaningrum *et al.,* 2003). Routine and proper cleaning of steel mesh gloves and other equipment should thus be mandatory. It would also be advisable to educate the meat handlers during seminars or workshops regarding the fact that surfaces should be washed with detergent and water and rinsed before being sanitised in order to remove

biofilms. The cleaning and sanitation process is very important, as it has been found that sanitisers can be neutrilized by soiled surfaces or organic materials (Gill and Jones, 1999; Martínez-Tomé *et al.*, 2000). The choice of the sanitising agent is equally important as it has been reported that *Staphylococcus* species are to a undesirable extent resistant to, for example, quaternary ammonium – based compounds (Heir *et al.*, 1999).

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CHAPTER 5

CONCLUSIONS

In recent years *Staphylococcus* species have been isolated from various food production environments and food products and their presence in the food industry is of special concern due to their potential to produce enterotoxins. Staphylococcal enterotoxins (SEs) are the second most common cause of reported foodborne illnesses. Although enterotoxin production was uniquely associated with coagulase-positive staphylococci (CPS) such as *S. aureus,* several species of coagulase-negative staphylococci (CNS) have meanwhile been found to be enterotoxin producers (Heir *et al.*, 1999; Balaban and Rasooly, 2000). Although it can be argued that, because fresh red meat will most likely undergo a form of heat treatment (grilling, baking, boiling, frying, etc.) one has to keep in mind that high temperatures will easily destroy the vegetative cells of bacteria. Heat-stable toxins such as those from *Staphylococcus* are likely to survive the heating process and still cause severe gastro-enteritis. With South African abattoirs increasingly looking to export to neighbouring countries more emphasis will be placed on the levels of contamination of the product when packaged and the shelf life of the product under environments such as vacuum. It was therefore the aim of this study to investigate the spoilage patterns of selected spoilage indicator microbiota (TVC and staphylocooci) under vacuum-packaging conditions as well as to investigate the origins of spoilage of such organisms from environments such as surfaces and the air in a highly active area such as the deboning room.

5.1 CONCLUDING REMARKS ON CHAPTERS 2, 3 AND 4

In Chapter 2, occurrence of *Staphylococcus* species in fresh vacuum-packed red meat that has been exposed to 5°C and 18°C respectively is discussed. The objective of the study was to assess the rate of survival of the various species if the product were to be exposed to inappropriate storage conditions. Chapter 3 reports on the occurrence of *Staphylococcus* species on the red meat prior to the vacuum-packing process in the deboning room of the high throughput abattoir, taking into account the corresponding bioaerosols present in this environment. Once the *Staphylococcus* species were quantified, they were then identified in order to determine the different species present as well as the sources of the various species. Chapter 4 reports on the final phase of the study, where the presence of staphylococci on the hands of meat handlers, their aprons, on working surfaces used for cutting and trimming the meat, and on the conveyor belt was assessed. The purpose of this chapter was to investigate which *Staphylococcus* species were present on the various surfaces and to determine which species adhered to the surfaces.

It was reported in Chapter 3 that staphylococci were present at relatively low levels on the meat and in the working environment, compared with the results of Schlegelová *et al.* (2004), where a definite increase of staphylococci levels was observed during carcass processing. Although relatively low numbers of staphylococci were observed, nine different species were identified, of which five are able to produce enterotoxins (Jay 2000). Table 5.1 provides an at

glance view of *Staphylococcus* species isolated in this study. In total, fourteen *Staphylococcus* species were identified of which 13 are known enterotoxin producers while *S. simulans* is known to be a non-producer of enterotoxins (Jay, 2000). The sources of the different staphylococcal species, as identified in the various environments, point to recontamination and / or cross-contamination of the product by the meat handlers (Desmarchelier *et al.*, 1999).

An interesting finding made in this study was the isolation of the following eight species: *Staphylococcus aureus; S. epidermidis; S. capitis; S. auricularis; S. hominis; S. saprophyticus; S. haemolyticus* and *S. simulans.* All of these species were present in the air, meat and surface environments of the deboning room whilst the majority are known to be indigenous to humans (Ieven *et al.*, 1995). This could suggest that the airborne staphylococci expelled by meat handlers settled both on the meat and on the working surfaces. Because of further contact with the product by the meat handlers, more staphylococci were likely to be deposited onto the meat. The species isolated in Table 5.1 indicate that the meat had an 85% likelihood of being contaminated with staphylococci from the surfaces within the deboning process as shown in Figure 5.1. The various surfaces would include the hands of the workers, the steel mesh glove, the aprons, the stainless steel tables and finally the conveyor belt, due to the manual processing of the product. The airborne staphylococci, on the other hand, had a 66% likelihood of contaminating both the surfaces and the air during the deboning process.

Table 5.1 *Staphylococcus* species isolated from various environments in the deboning room of a high throughput red meat abattoir

***Staphylococcus* species**	**Enterotoxin**	**Environment**		
		A	**B**	**C**
Staphylococcus aureus	+	✔	✔	✔
S. epidermidis	+	✔	✔	✔
S. capitis	+	✔	✔	✔
S. auricularis	+	✔	✔	✔
S. hominis	+	✔	✔	✔
S. saprophyticus	+	✔	✔	✔
S. haemolyticus	+	✔	✔	✔
S. simulans	-	✔	✔	✔
S. sciuri	+		✔	✔
S. intermedius	+		✔	
S. xylosus	+		✔	✔
S. cohnii cohnii	+		✔	
S. lugdunensis	+		✔	✔
S. warneri	+			✔
Total species isolated: 14		8	13	12

Note: + = positive; - = negative; ✔ = presence; A = air; B = Meat; C = Surfaces
A = Species as described in Chapter 3
B = Species as described in Chapters 2 and 3
C = Species as described in Chapter 4

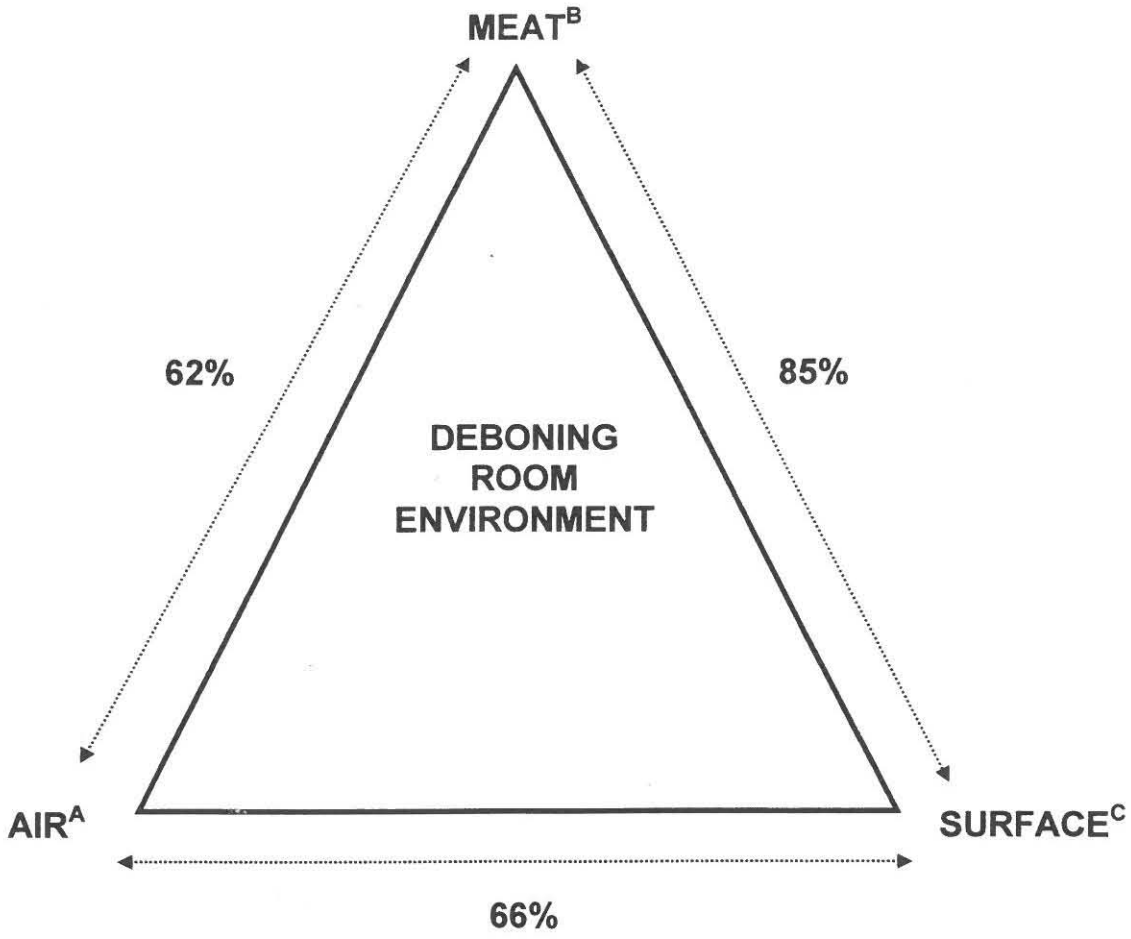

Figure 5.1 A proposed diagram indicating the likelihood of staphylococci contamination via the air, surfaces and meat in the deboning room (A isolates / B isolates x 100 = 62%; B isolates / C isolates x 100 = 85%; A isolates / C isolates x 100 = 66% as shown in Figure 5.1.

The ability of staphylococci to adhere to surfaces could also explain the presence of these species on the meat (62%) likelihood.

Considerable variation was found throughout Chapter 2, 3 and 4 in terms of the various environments sampled as well as the microbiota enumerated. These variations suggest definite inconsistency in, and absence of, good manufacturing practices by the meat handlers. At 18°C staphylococcal counts showed levels as high as 10^5 CFU.g^{-1} after a 48-hour exposure to this temperature. Apart from presenting a definite health hazard, these high levels would definitely have a negative effect on the quality and sensorial acceptability of the product. The majority of the species identified on the product and in the air were also present on the working surfaces. This is a probable indication of organisms being harboured by meat handlers as well as on the various surfaces due to inadequate cleaning processes. It has been reported in literature that the effectiveness of disinfectants, even when used repeatedly, cannot always be relied upon, especially when the cleaning processes result in minor cuts, abrasions and wounds to the hands of workers.

The results reported in Chapter 4 indicate that the various surfaces were moderately contaminated ($\bar{x}$ <10 CFU.cm^{-2}) with staphylococci species. An important observation that was made during sampling of the various surfaces was seemingly the inconsistency in sterilisation of working equipment and surfaces between carcass-breaking processes. It was furthermore noted that the workers did not wash or sanitise their hands between carcass-breaking

processes. This could, to an extent, explain the high prevalence of *Staphylococcus aureus* on the hands and gloves of the meat handlers. The occurrence of staphylococci could also be attributed to the lack of supervision with regard to the hygiene practices of workers in the deboning room as well as the lack of screening for *Staphylococcus* species associated with the meat handlers themselves. The high levels of staphylococci noted in Chapter 2 could also be related to the moderately contaminated surfaces as described in Chapter 4. Both coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS) isolated during the course of this study are of particular concern to the meat industry as both pose a possible risk of causing foodborne infections when present at high levels.

5.2 RECOMMENDATIONS TO INDUSTRY

It is of paramount importance that the meat being processed be as free as possible from microbial and foreign body contamination. It is also evident that contamination may arise from the processing environment, which includes food contact surfaces, the air and people. Failure to control these factors may ultimately lead to product recalls, loss of sales or profits and poor publicity. The aim of each perishable food related industry should be to produce food products that meet in-house specifications and that conform to local regulations. Apart from this, the product should also meet the consumer's needs, be safe, wholesome and nutritious.

With regard to the results found in this study the following specific recommendations are made to industry:

- The success of an organised hygiene, sanitation and food safety program largely depends on the commitment of management. Management should therefore participate actively in the development of the above-mentioned programs. It is furthermore management's responsibility to select capable and trained employees to carry out tasks, to provide adequate equipment as well as to budget accordingly in order to accomplish the goals of the company so as to maintain the standard and quality of the final product.

- Special attention should be given to personal practices of meat handlers, especially those on duty in the deboning room. This is important because meat handlers who cough and sneeze while handling meat, who do not wash their hands frequently and who are careless and ignorant with regard to personal and process hygiene, can transmit staphylococci into the processing area.

- Meat handlers wearing gloves should wash their hands on a regular basis as *Staphylococcus aureus* has been shown to accumulate as hands perspire and may multiply on hands encased in gloves for long periods. The steel mesh gloves should be washed and cleansed at regular intervals during processing to help prevent the accumulation of bacteria that could lead to further cross-contamination of the product.

- Meat handlers should wash and rinse their hands thoroughly with detergents and hot water, as this removes many transient foodborne pathogens. To improve the effectiveness of hand-washing, application of appropriate antiseptics during or after washing is recommended, together with creams containing antiseptics.

- Hair covers should be worn at all times because hair from the head, face or arms is a probable source of contamination by *Staphylococcus* species and other bacteria.

- Facial masks should be worn during processing, as the deboning area is a critical handling operation. Nasal cavities are laden with staphylococci and these organisms are continuously expelled from the nose and mouth of individuals.

- Meat handlers should undergo pre-employment as well as periodic medical examinations. These should include physical examinations and reports of medical histories. Screening for staphylococci should be done on a regular basis and personnel should be instructed to report any infection, no matter how seemingly unimportant, to the resident occupational nurse or medical officer.

- Knowledge through proper training is the basis of an effective food safety program. It is important that the meat handlers and supervisors receive

appropriate training on aspects such as good manufacturing practices, proper food handling techniques and basic food protection principles.

Another vital process is the proper sanitation of all surfaces and equipment. Sanitation is the process intended to remove all undesirable material from surfaces to a level at which any remaining residues present a minimal risk. As shown in Figure 5.2 a proper sanitation procedure consists of five essential steps: The preparation step of the sanitation program will include dismantling of equipment, removing of ancillary equipment and removing of gross soils or pieces of meat stuck within the equipment. Step 2 should be carried out as soon as possible after processing has ceased, since "dried on" soils become increasingly difficult to remove. The principle goal of this step is to loosen or remove as much gross soil as possible from the surfaces to allow the cleaning solution improved access to the more difficult deposits that need to be removed. In the abattoir studied, the conveyor belt, was especially heavily soiled with pieces of meat, which allowed the bacteria to adhere to the grid surface of the belt. Because of the one-way conveyor belt system it would also be advisable that the belt be cleaned with high-pressure water so that the morning session's soil can be loosened and removed before the afternoon session commences.

It is furthermore advisable that the water temperature be 38-46°C for both pre- and post-rinsing. Where fatty soils are encountered, a rinse temperature above the melting point of fat should be used (usually 45-50°C). Water temperature control is essential throughout the cleaning process and should

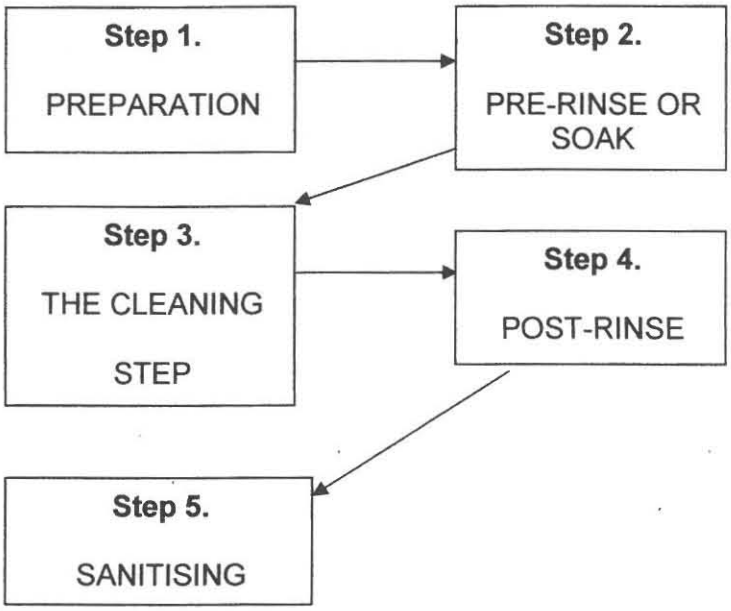

Figure 5.2 A schematic flow diagram illustrating the five steps of a proper sanitation procedure

be determined by the type of soil present. Step 3 (Cleaning) introduces a detergent solution with good wetting and penetrating properties and some form of mechanical action applied by spraying, flushing, brushing or rubbing. The post-rinse step accomplishes the removal of loosened soil and detergent residues by warm water rinsing to prevent re-depositing of the soil, leaving clean surfaces ready for sanitising. The final step of sanitising or disinfecting will reduce the number of microorganisms present and will eliminate their one essential need for life – food. Sanitising can be accomplished either by soaking the item for at least 10 minutes at 80°C or through the use of a chemical sanitiser. The high temperatures help to destroy bacteria when applied for longer time periods.

Sanitisers should be allowed to remain on work surfaces overnight. Clean work surfaces and equipment should also be sanitised directly before use. Finally, to determine whether the cleaning programme followed is correct and whether the chemical sanitiser in use is effective, areas that are exposed to foods can be swabbed. Analysis of swabs will show the presence of any bacteria. Such analyses can be done to genus and species level to assess specificity of the sanitiser towards the various microbiota. In terms of staphylococci, this will allow the selection of sanitising and detergent agents specifically aimed at reducing the numbers of these organisms. From the results, an action plan can be worked out. This is of the utmost importance as several staphylococci have been found to be resistant to quaternary ammonium compounds (QACs) (Heir *et al.*, 1999).

Finally, it would be advisable that the supervisors draw up a detailed cleaning schedule (including a master-cleaning schedule) that would include daily, weekly and monthly cleaning schedules. This schedule should be compiled and closely monitored to ensure that every item of equipment and every surface is maintained in a hygienic state and free of harmful microbiota. The cleaning schedule will be able to answer questions such as who, where, what and when activities are being carried out. This includes the time each item is to be cleaned, who is responsible for accomplishing the cleaning, and, as the cleaning becomes accomplished, the schedule becomes a record of the work completed (Ramphal, 2004).

In conclusion the consumer expects that his/her food supply be nutritious, wholesome and safe. It is the responsibility of the food industry to conform to the standards and specifications stipulated by the local health regulations. With increased possibilities for export the majority of abattoirs in South Africa are working towards the implementation of a HACCP system and the success thereof largely depends on the establishment of good manufacturing practices. The specific abattoir investigated in this study should pay attention to personnel practices which should include training on personal and process hygiene as well as cleaning and sanitation. The success of these programmes will ultimately determine the quality of the product.

5.3 FUTURE RESEARCH

With regard to this study, further research opportunities would include the following:

- further studies on the occurrence of bioaerosols and microorganisms that may arise from the ventilation system of the deboning room;
- studies on the presence of pathogens on carcasses prior to entering the deboning room;
- studies on the occurrence of staphylococci on meat handlers during the different seasons to establish which species would be more dominant;
- an expanded study on the effectiveness of sanitizers used in the cleaning and sanitation procedures of a deboning room;
- an investigative study on the knowledge of workers concerning HACCP and the importance thereof especially in the meat industry.

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APPENDIX A

Figure 5.3 Illustration of a typical deboning process

Figure 5.4 An example of a vacuum-packaging machine used in a deboning plant

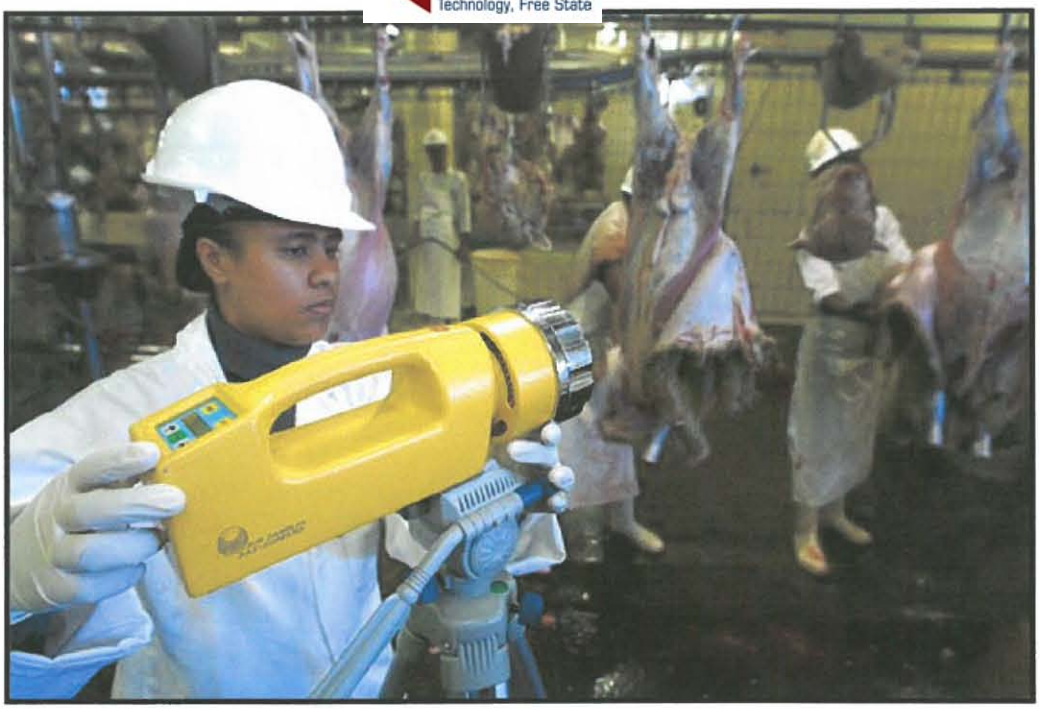

Figure 5.5 A picture of the researcher operating the SAS Super 90 microbial air sampler

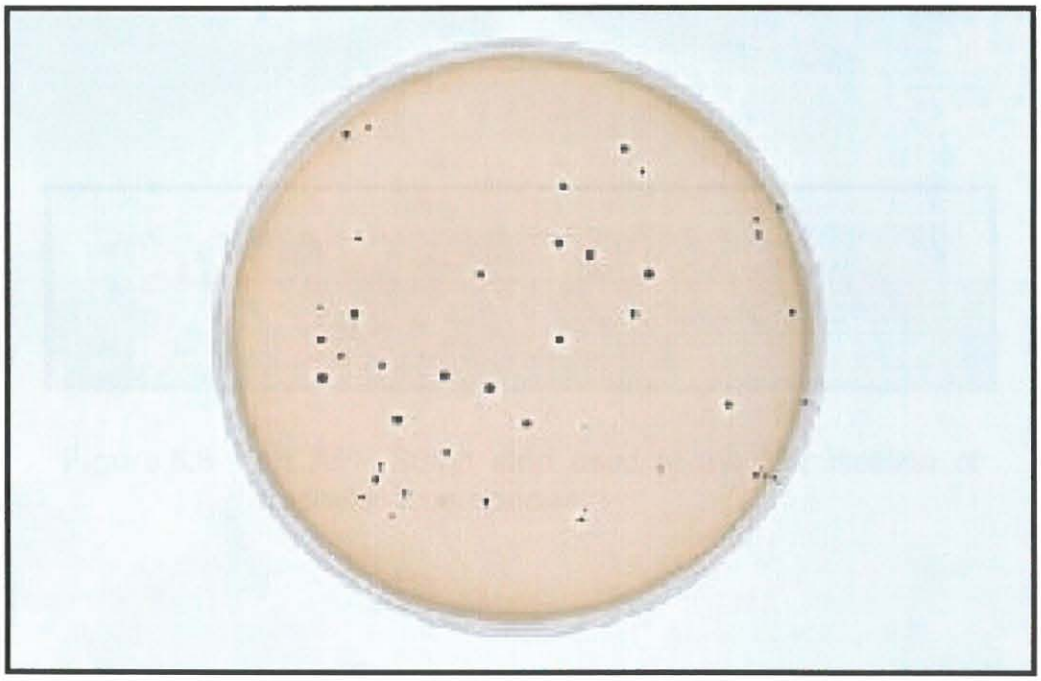

Figure 5.6 *Staphylococcus* colonies on Baird-Parker Agar

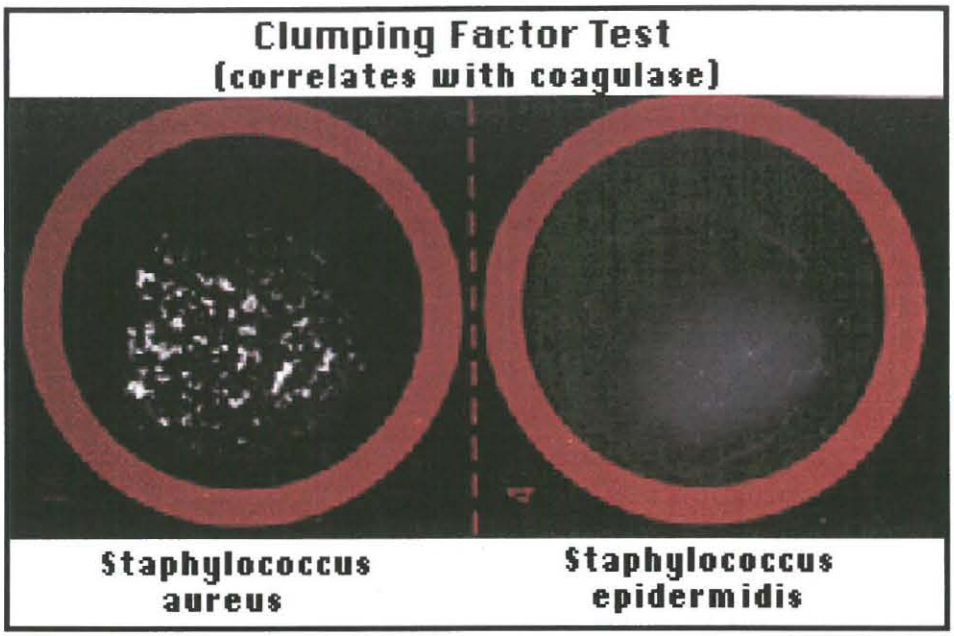

Figure 5.7 An example of a coagulase-positive (CPS) and coagulase-neagtive (CNS) staphylococci

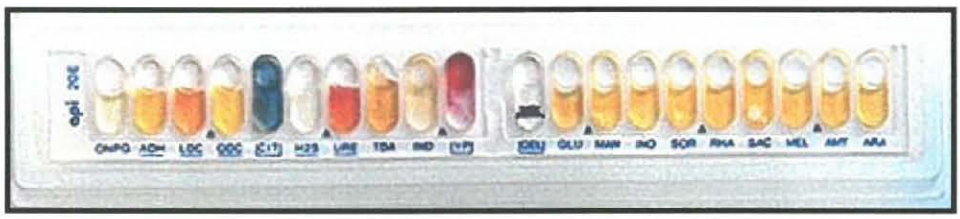

Figure 5.8 An API- Staph strip used in the identification of staphylococcal species

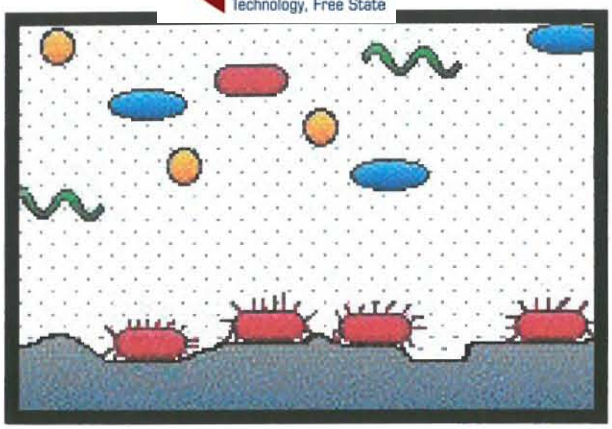

Figure 5.9 A schematic of the development of a biofilm

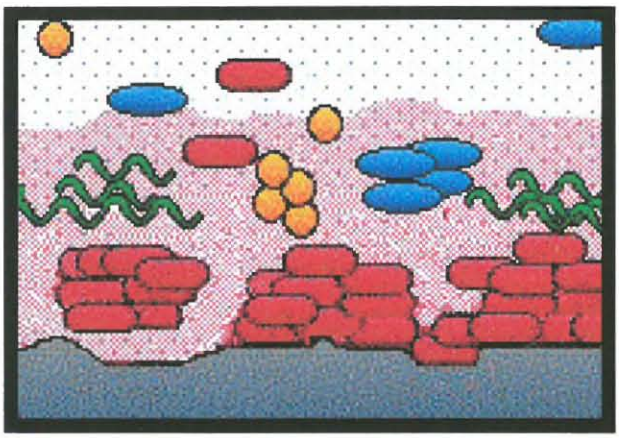

Figure 5.10 Shows the initial adhesion involving the host cell surface

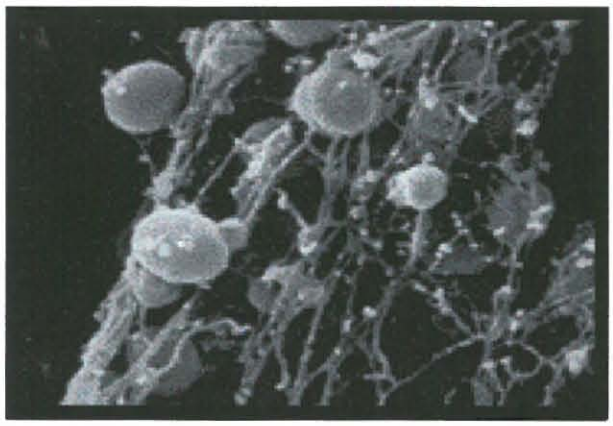

Figure 5.11 A scanning electron micrograph of a biolayer