MICROBIAL HAZARDS ASSOCIATED WITH MEAT PROCESSING IN BUTCHERIES WITHIN MANGAUNG METROPOLITAN MUNICIPAL AREA

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DECLARATION OF INDEPENDENT WORK

I, the undersigned, do hereby declare that this research project submitted to the **Central University of Technology, Free State**, for the degree **MAGISTER TECHNOLOGIAE ENVIRONMENTAL HEALTH: FOOD SAFETY** is my own original and independent research work that is true and authentic. This research work has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for attainment of any degree or qualification.

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LEBOGANG BRENDA SHILENGE **DATE**

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SUMMARY

In the battle to sustain and produce quality food that is safe and affordable, the limited legislative and regulatory environment continues to allow opportunities for food to become contaminated during processing. The degree of contamination distributed over the final food product (including meat products) depends upon several factors that include knowledge and behaviour of the food handlers, equipment, the hygiene habits of personnel, and the monitoring that takes place at food processing plants (including butcheries).

The current study was conducted in five selected butcheries (forming 15% of the registered butcheries at the time the study was conducted) in the Mangaung Metropolitan municipal area, purposely targeting the ones registered with the municipality. The hygiene practices of meat handlers were assessed (through self-administered questionnaires) because meat is a perishable product that requires labour intensive processing for production of quality products. Thus, mishandling by food handlers may create and maintain conditions favourable to microbial contamination. Furthermore, the study assessed and characterised microbial contamination on working surfaces and utensils through swabs as well as bioluminescence instrument [Adenosine Tri-phosphate (ATP) Hygiena] for cleanness of the working environment. Concomitant to the above, meat handlers' hands and aprons were also assessed for possible microbial contamination as well as their characterisation. Lastly, aerosolised microbes [through an air sampler (Surface Air System) SAS Super 90] were also collected for

quantification and identification during working hours as airborne microbes can settle on working surfaces and/or utensils as a result of movement of workers and other related working processes. Statistical points such as correlations, standard deviations, group standard deviations as well as significant differences were captured per respective chapter where necessary. Data reported in this study is over 3 month period with two weeks intervals during sampling and thus reported as either weekly or rounds between sampling periods.

The results of the current study indicate that the food safety objectives are negligibly achieved, indicating a need for proper food safety training which is audit based. On administration of a questionnaire, food handlers showed poor knowledge of food safety awareness coupled with poor attitude and behaviour in terms of food safety. The five butchery premises were further examined regarding the airborne and surface microbial loads, as well as that of the food handlers' hands, during processing. The microbial loads in the air appeared to comply with the suggested limits at all the sampled butcheries. Microbial loads on meat contact surfaces showed levels conforming to the South African standard or guideline of 1×10^2 cfu.m^{-2}. Total Coliforms on hands and on aprons were compared to the general microbial target value of <2.5 cfu.m^{-2} as suggested by literature.

In this study, Matrix Laser Desorption Time of Flight Mass Spectrophotometer (MALDI-TOF MS) was found to be an accurate, rapid and cost effective method towards

identifying of foodborne pathogens and spoilage bacteria including yeast. Moreover, in recent years South Africa's meat scandals have increased consumer awareness and the demand for food safety. Section 11 of the Meat Safety Act (Act no. 40 of 2000) stipulates that every abattoir must utilize an independent inspection service appointed by the department of agriculture to ensure that meat of high quality and wholesomeness is produced. However, once the meat and meat products leave the abattoir, they are under the jurisdiction of the local authorities who rely only on visual assessment as opposed to microbiological inspection in the maintenance of their hygiene and quality. Despite the high incidence of foodborne illnesses in both developed and developing countries; South African data on foodborne illness incidents is still insufficient. This could be attributed to the fact that in South Africa, legislation governing the acceptable standards of the levels of microbiota in the air and on food handlers' hands is still inadequate. Additionally, lack of obligatory usage of Hazard Analysis Critical Control Point (HACCP) procedures in the meat premises poses a risk for economic productivity.

In conclusion, the identification of airborne bacteria in the butcheries strongly suggests that in the planning of the existing establishments, the building layout, control of the traffic flow of personnel, the durability and imperviousness of floors, the ventilation system and the placement of the equipment were not carefully considered. This may play a role in the prevalence and proliferation of airborne microbes as the resulting establishments provide an environment conducive to the breeding of microbes.

In regard to swabs, it was concluded that floors may present a high point of contamination possibly through aerosolization of microbial communities. Moreover, cleaning materials and hygiene practices need to be reviewed. The results of the administered questionnaire showed that food handlers should be sufficiently trained with regard to food quality management tools such as Good Manufacturing Practices (GMP), Hazard Analysis and Critical Control Point (HACCP) systems and food safety. The evaluation of meat contact surfaces for organic soils to determine their cleanliness using the rapid ATP bioluminescence testing can be convenient for everyone involved in the food chain since visual and touch inspection cannot be conclusive enough to meet regulatory requirements in terms of microbial counts.

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MICROBIAL HAZARDS ASSOCIATED WITH MEAT PROCESSING IN BUTCHERIES WITHIN MANGAUNG METROPOLITAN MUNICIPAL AREA: GENERAL BACKGROUND

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1.1 Backdrop to the South African traditional and retail butcheries

The safety of meat remains one of the major priorities on the agenda of most meat producers, processors and consumers. This is due to a number of highly publicized food scares and outbreaks worldwide such as Bovine Spongiform Encephalophathy (BSE), avian flu as well as foot and mouth disease including emerging or evolving pathogenic bacteria such as *Escherichia coli* 0157:H7 and *Listeria monocytogenes* (Sofos, 2008; Seeiso, 2009). Despite the current number of South African meat export and numerous food safety education that food business operators and food handlers receive, foodborne illnesses resulting from the consumption of contaminated meat is still a continuous public problem in developed and developing countries including South Africa (Redmond and Griffith, 2003; Griffith, 2006; Jacob *et al.,* 2010). In general, it is crucial to improve the "farm to fork" concept in order to prevent, or at least control, major problems associated with foodborne diseases related to meat products (Jacob *et al.,* 2010).

In order to address contamination challenges in the meat industry, one of the most crucial steps in a meat hygiene system is the monitoring of all aspects of animal husbandry practices on the farm, and the move towards the production of safe and healthy livestock. Farm animals are the original source of some of the foodborne pathogens that cause diseases in humans as they show no symptoms of illness although they faecally excrete pathogens (Ateba *et al*., 2008; Blackburn and McClure, 2009; Behravesh *et al*., 2012). To avoid a high level of cross contamination, farmers and veterinary practitioners are responsible for ensuring that only animals suitable for

loading, travelling and subsequently unloading are transported to abattoirs (Nørrung and Buncic, 2008). Animals are then sent to abattoirs for slaughter where processes are regulated by the South African Meat Safety Act (Act 40 of 2000) as well as other local and international regulations. The carcases from abattoirs are then transported to the butcheries by means of cold trucks, where they will be offloaded and kept at the required temperature, then processed, packaged and labelled.

1.2 Typical layout of butcheries

The Butcher (2014) reports that R.918 (Regulation 918) as promulgated by the South African Health Act, (Act no. 63 of 1977) details the requirements' of a butchery setup in relation to other food premises; however, at the butchery, sources of contamination can be anything that directly or indirectly comes into contact with the meat. Therefore, it is recommended in the South African Meat Safety Act (Act no. 40 of 2000) under the Department of Agriculture that the processing facility and its structure, including walls, ceilings, floors, windows, doors, vents, and drains, should be designed in a manner that makes it is easy to clean and maintain, as well as to protect the product from possible microbial, physical and/or chemical contamination. In the traditional butcheries, the entrance for customers is normally directly facing the display area (personal observations as adapted from studied butcheries; Shilenge, 2011). The entrance for meat handlers is provided with a hand-washing basin, soap dispensers and a hand-drying facility (Figure 1.1 illustrates a typical township butchery layout with regard to the setup only).

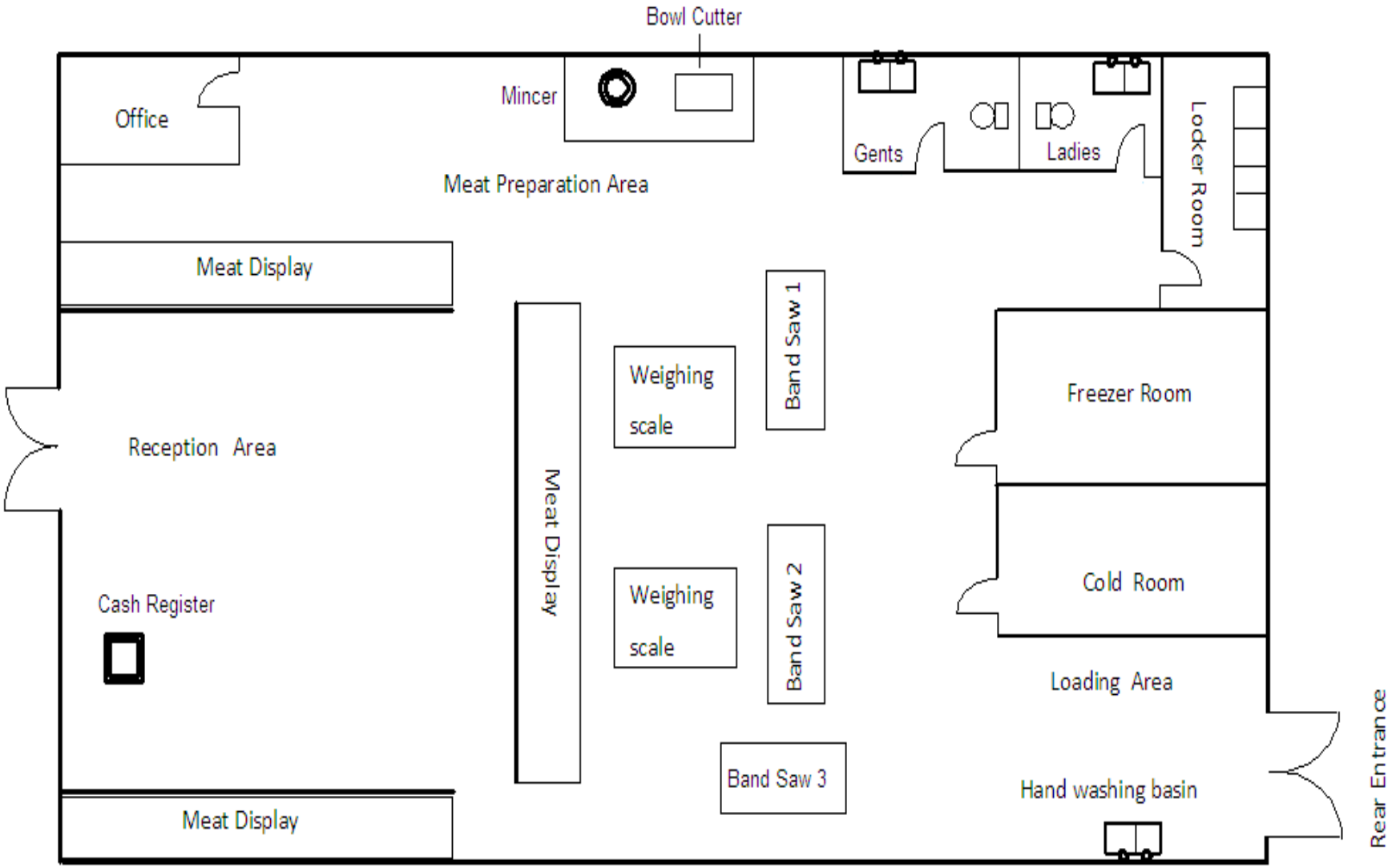

Figure 1.1: A typical schematic representation of a floor plan of butchery in a typical township (adapted from studied butcheries; Shilenge, 2011)

The change room is also usually provided, with a shower, hand-wash basin and lockers. Moreover, clean tables and chairs are found in the canteen for persons working in the butchery. An office is normally allocated to the manager and the shift supervisors. In addition, the floors and walls of the butchery building should be smooth, impervious and washable, and drains should have gratings or covers. At the point of sale, there is usually one or two display fridge units which carry vacuum packed and unpackaged meat which is ready to be weighed for customers and covered at all intersections. Figure 1.1 also elucidates where the meat processing equipment and fridge displays, freezer rooms and cold rooms would be situated (personal observations as adapted from studied butcheries; Shilenge, 2011).

1.3 Possible sources of contamination

1.3.1 Raw meat

The muscle tissue of a healthy living animal is free of microbes and the carcass meat under the skin is regarded as sterile immediately after slaughter (McEvoy *et al*., 2000). Contamination of meat may be due to slaughtering of stressed animals, as well as contact with external surfaces such as hair, both the gastrointestinal and respiratory tracts. In the abattoir, contamination occurs with the microorganism's introduction to direct meat contact surfaces in operations performed during offloading, weighing, processing, cutting and storage, as well as at the points of sale and distribution (Nørrung and Buncic, 2008; Sofos, 2008; Ali *et al*., 2010). Typical microorganisms that are usually prevalent in raw meat includes *Listeria monocytogenes*, *Salmonella,*

Staphylococcus aureus, *Campylobacter* (on poultry), *Escherichia coli* and *Escherichia coli* 0157:H7 amongst others (Forsythe, 2000; Insausti *et al*., 2001; Ateba *et al*., 2008).

1.3.2 Food handlers and related hygiene practices

Meat cutting is of paramount importance in meat processing as carcasses are deboned and cut into smaller and more desirable cuts using hand tools and machines (Wang and Shanmugam, 2009). Workers' physical effort is required for the traditional techniques including the use of both hands – one hand to hold the meat while the other hand uses the saw or knife. According to Nørrung and Buncic (2008), the process of meat handling increases the possibility of microbial contamination because unhygienic practices during handling may lead to transmission of bacteria to the meat from the surfaces. Several studies have further indicated that foodborne illnesses occur due to poor handling of food (Van Tonder, 2004; Griffith, 2006). *Staphylococcus*-related food poisoning has been linked to food handlers who are known to be carriers of this bacterium in meat establishments (Van Tonder, 2004).

Furthermore, lack of proper food hygiene practices has been reported to lead to contamination as a result of unclean hands after visiting the toilets. Moreover, through cross contamination from raw meats to salads via hands of food handlers. In addition, 97% of food handlers' illnesses in the USA were linked with improper food handlers' practices in the food-service industry (Bas *et al*., 2000). Bas *et al*. (2000), further stated that pathogens are passively transmitted from a contaminated source such a raw poultry to cooked food such as prepared for later consumption as colds foods. Food

handlers may on some occasions serve as sources of contamination especially as a result of some having gastrointestinal illnesses or convalescence process when symptoms have disappeared.

1.3.3 Transportation

Good quality meat with an adequate shelf life can be ensured by the proper maintenance of the cold chain. The South African Regulation 918 of 30 July 1999 framed under the Health Act, 1997 (Act no. 63 of 1977), National Health Act 61 of 2003 and the Meat Safety Act 2000 (Act no. 40 of 2000) clearly reflects and stipulates that all food specified under the regulation and act must be kept at a low temperature (4ºC) during storage, transport and while on display. Additionally, no food may be transported simultaneously with any person or items, or in such a manner that it comes into contact with the floor or anything else that can pollute, spoil or contaminate the meat in anyway (Van der Walt, 2005). Thus, inspection of incoming meat and temperature checks of both the meat and transport used are of principal significance as described in the South African Regulation 918 of 30 July 1999 under the Department of Health (DOH).

1.3.4 Bioaerosols

The microbial contamination of meat and meat products in the past was thought to occur only when such products came into direct contact with contaminated surfaces. However, airborne microorganisms, dust, pollen and mould spores which may be present in ambient air, are contaminants which can easily find their way into the products (Sutton, 2004). These airborne contaminants are also generally known as

bioaerosols, and may include bacteria, fungi, viruses, pollen, toxins and other contaminants of non-biological and biological origin (Shale *et al*., 2004; Nkhebenyane, 2011). Several studies have indicated a range of routes by means of which microorganisms can be distributed through the air such as talking, sneezing, coughing and high pressure spraying (Cundith *et al.,* 2002; Shale *et al*., 2004; Sutton, 2004; Van Tonder, 2004). Furthermore, wastewater, sink and floor drains, including spilled products that become aerosolized, can also be major sources of bioaerosols causing harm to both the consumer and worker's health, possibly leading to the reduction of the shelf life of meat and meat products. The use of air filtration is of vital meaning to ensure fine quality of air in high risk areas such as the preparation and packaging areas, as well as at the purchasing point (Patel, 2009). However, such methods do not necessarily stop the distribution of bioaerosols in food processing areas.

1.3.5 Biofilms

Biofilms are generally described as microbial populations (mainly bacteria) that have the ability to adhere to different surfaces. They are also Extracellular Polymeric Substance (EPS) producers, which are highly hydrated with chemically complex matrix (Donlan, 2002; Hall-Stoodley *et al*., 2004). The characteristics of EPS are indicated as a reason for the resistance of treated biofilms to sanitizing, rather than intrinsic attributes of the cells in the biofilm (Pan *et al*., 2006). Studies have illustrated that common sanitation practices are less effective in removing biofilms as compared to free cells (Meyer, 2003). The leading causes of the nosocomial infections in the USA, among other countries, are biofilm-related infections sourced by staphylococci (Kong *et al*., 2006).

Studies have also shown that, as in other food sectors, the meat industry is faced with increasing demands in terms of cleaning and disinfection in order to remove microbial coatings such as biofilm which may take days or hours to form (Stopforth, 2002).

1.3.6 Equipment and utensils

Even with hygienic design features, equipment may still become contaminated by microorganisms, workers, bioaerosols and other materials during processing (Evans *et al.,* 2004). Many foodborne disease outbreaks are associated with improperly cleaned utensils and equipment. According to Gill and McGinnis (2000), meat residues that are not removed on meat contact surfaces during cleaning have been indicated to be the primary source of *Escherichia coli* deposited on the meat. *Listeria monocytogenes* is an environmental bacterium which can harbour and thrive in meat processing equipment such as slicers, dicers and machinery for packaging, which are insufficiently cleaned and sanitized (Tompkin, 2002; American Meat Institute, 2008). Table 1.1 gives a list of common equipment that is used in the butcheries and the typical microorganisms associated with this equipment.

1.3.6.1 ATP (Adenosine Tri-Phosphate) Hygiena

The formation of biofilms on equipment and/or utensils can be of great concern in the meat industry. Hence, with the above in mind, it is crucial to take note that there has been the use of visual inspection in most food premises to check equipment used and working surfaces. ATP Hygiena amongst others, has been used to evaluate the cleanness of working surfaces where Surface Adenosine Triphosphate (ATP) which is

Table 1.1: Equipment and utensils commonly used in butcheries

Equipment and utensils	**Uses**	**Prevailing micro-organisms**	**References**
Knives	Used for deboning, cutting, slicing and dicing.	*E. coli and L. monocytogenes*	Rivera-Betancourt *et al*., 2004
Bandsaws	Sawing through tough muscles, carcasses and cutting of frozen meat.	*Salmonella, E. coli and L. monocytogenes*	Warriner *et al*., 2002
Bowl cutters	Chops meat into small pieces, thus finely mincing meat, blending and emulsifying proteins.	*S. aureus*	Downes and Ito, 2001
Chopping boards	Used to slice meat.	*Salmonella*, *S*. a*ureus*, *P. aeruginosa* and *Clostridium* spp.	Ak *et al.,* 1994
Meat slicers	Used mainly for cutting ready-to-eat meat into desirable slices.	*L. monocytogenes*	Meat Safety Act, 2000 (Act 40 of 2000); Blackburn and McClure, 2002; American Meat Institute, 2008
Meat grinders	Minces the meat through different discs to a desirable size. Grinding employs torque –a force producing a twisting effect.	*L. monocytogenes*	
Cold room	Used to store chilled meat to prevent growth of microorganisms.	*L. monocytogenes*	
Freezer room	The operating temperature should be -18ºC for freezing the meat.	*L. monocytogenes*	

an enzyme that is present in all living cells, can be detected thus reflecting the amount of organic matter that remains after cleaning an environmental surface. Most hospitals have employed the use of ATP-based sanitation monitoring systems to detect and measure ATP on surfaces as a method of ensuring the effectiveness of their facilities' sanitation efforts. The amount of ATP detected, and where this ATP was detected, indicates areas and items in the healthcare setting that may need to be re-cleaned, and the possible need for improvement in a healthcare facility's cleaning protocols. This can also be applied in the food industry as some companies have started doing so although this system is not widely used in South Africa.

As stated before, the primary monitoring of any cleaning programme is visual cleanliness, it involves the assessment of a surface as being free from food debris and other soiling by a person without any sampling aids, example. This may involve looking at the surface, feeling the surface for any signs of invisible deposits such as grease, oils and even smelling the equipment. In Egypt as an example, most local health departments utilise visual but not microbiological methods when inspecting hygiene status of butcheries area in small scale processing plants (Attala and Kassem, 2011) and the use of ATP Hygiena is still lacking and not well documented in areas such as butcheries.

1.3.7 Public health disease surveillance system and related pathogens

1.3.7.1 Public health disease surveillance system

In South Africa, food poisoning became a notifiable medical condition in 1990; however, the condition is less likely to be reported due to lack of efficient and integrated foodborne surveillance systems (South Africa, Department of Health, 2007). However, internationally, CDC's (Centre for Disease Control) National Notifiable Diseases Surveillance System (NNDSS) utilises a multifaceted Public Health disease surveillance system that gives public health officials powerful capabilities to monitor the occurrence and spread of diseases. This section of CDC is used by numerous state, territorial, tribal, and local health departments; and by partner organizations, such as the Council of State and Territorial Epidemiologists (CSTE), to facilitate collecting, managing, analysing, interpreting, and disseminating health related data for diseases designated as nationally notifiable. Moreover, develop and maintain national standards applicable across states, maintain the official national notifiable diseases statistics. Furthermore, provide detailed data to CDC programs to aid in identifying specific disease trend, work with states and partners to implement and assess prevention and control programs, and publish summarized data findings weekly and annually in the Morbidity and Mortality Weekly Report (CDC, 2014).

Unfortunately, South Africa lack such a structure and there is a dire need as this system is an effective public health surveillance that must begin at the local- and state-health department levels. Government must work with a variety of healthcare providers, including laboratories, hospitals, and private providers, to obtain case reports on many

infectious and some non-infectious diseases. Each province must have by laws mandating that providers report cases of certain diseases to province and/or local health departments (CDC, 2014; South Africa, Department of Health, 2007).

1.3.7.2 Pathogenic microbes of concern

The largest outbreak of *E. coli* 0157:H7 occurred in South Wales in 2005 where a total of 157 cases were identified. A hundred and eighteen of these cases were confirmed positive for *E. coli* 0157:H7 and 31 children in schools were admitted to hospital. One death (of a 5-year-old) was reported after consumption of sliced cooked meat and other types of meat supplied by John Tudor and Sons, a catering butchery business (Pennington, 2009; Powell *et al*., 2011).

On the other hand, *Listeria monocytogenes* was reported to have caused an outbreak of food poisoning after consumption of deli meats in Toronto butchery in 2008. The cause of this outbreak was mainly due to trapped meat residues in meat slicing machines which provided a reservoir for *L. monocytogenes* (Pennington, 2009). A decade earlier than the latter (during 1999), it was estimated that foodborne pathogens caused 76 million episodes of illness, resulting in 325,000 hospitalizations and 5000 deaths in the United States alone (Osterholm, 2011). The Centre for Disease Control and Prevention estimates that there have been approximately 48 million foodborne illnesses, 128,000 hospitalizations and 3000 deaths post 1999 until the 2011 (CDC, 2011).

In South Africa as in other countries as reported by Powell *et al*. (2011) and Halliday *et al*., (2012); it remains a challenge to enforce regulations in some sectors due to the lack of surveillance data and in the absence of outbreaks. The implication of the above is that there was a substantial decrease in the estimated incidence of foodborne diseases between 1999 and 2010. However, as reported by Sofos (2008), the 1999 estimates cannot be compared with the current ones for purposes of trend analysis due to the fact that different diagnostic methods evolve all the time.

Furthermore, the epidemiological data of foodborne illness and surveillance estimated by the U.S. (CDC, 2011) such as Food Net and the pathogenic tracking and DNA fingerprinting program (PlusNet) indicated that approximately 60-70% of outbreaks and 40-50% of foodborne illness cases reported remains unresolved as well as the etiologic agent unknown. In addition, some pathogens of current concern were not known or were not suspected of causing foodborne illness in the recent past years such as *E. coli* 0157:H7, *L. monocytogenes*, *C. jejuni* and *Y. enterocolitica.* The number of pathogenic microorganisms that are new such as *E. coli* 0157 (emerging) and *L. monocytogenes* which are known but not associated with foodborne disease transmissions, or evolving microorganisms such as *Salmonella* have been associated with documented foodborne illness episodes and their numbers appears to be increasing (Sofos, 2008).

1.4 Legislation and governance concerned with South African butcheries

1.4.1 Food safety, hygiene regulations and legislation

There are laws and regulations in place to secure hygienic conditions and practices to protect the consumers against potential risks of food poisoning (Table 1.2).

1.4.1.1 The role of the National Department of Health

The Department of Health's responsibility is to make a contribution to protect South African people from harmful effects of unsafe foods. At a national level, food control directorate, incorporated in the Chief Directorate is directly responsible for all matters related to food safety control. Furthermore, Regulation R.908 of 2003 states that a business selling food to the public should have an HACCP system (South Africa, Department of Health, 2003). However, HACCP is not mandatory for local butcheries due to the reason that local by-laws do not require the use and/or implementation of HACCP, rather it is optional and used by butcheries at their own discretion (City of Johannesburg, 2014).

1.4.1.2 The role of the municipality (and EHP's) regarding the butchery

A butchery, by virtue of being a food premises, is required by law to observe all regulations governing food premises. It is for this reason that butcheries are required to display valid certificates of acceptability once they are in compliance. The certificate may be applied for and is obtainable from the local authority. During inspections of butcheries, Environmental Health Practitioners (EHP's) only conduct a visual routine

Table 1.2: Acts, regulations and standards governing butcheries in South Africa

ACT NUMBER, REGULATIONS AND STANDARDS	**TITLE**	**SUMMARY**
Act 54 of 1972	The Foodstuff, Cosmetics and Disinfectants Act	The act governs all foodstuffs manufactured, processed or sold in South Africa, including those imported into South Africa. In addition, the act requires producers to declare aspects such as food-related allergens and specific ingredients in the product, since consumers rely on the information on the labels to make sensible decisions when purchasing.
Act 61 of 2003	The National Health Act	Recommends factory conditions and hygiene for food handlers. Covers the storage, transportation, handling and processing of raw and finished products. Gives optimal storage temperatures.
Act 40 of 2000*	**The Meat Safety Act***	**In essence, section 12 of regulation R.918 places the responsibility on the butcher to ensure that in the butchery only meat derived in accordance with the Meat Safety Act is handled**.*
Act 68 of 2008	The Consumer Protection Act	Aims to protect and prevent consumers from consuming food products which are hazardous to their health.
SANS 10049:2012	Food Hygiene Management	Covers provisions for the hygienic handling of food and beverages for human consumption, in order to ensure a safe, sound and wholesome product.
Government notice R908 of 2003	Regulations relating to the application of the HACCP System	Specifies the requirements and application for hazards analysis critical control point, which are promulgated under the Foodstuffs, Cosmetics and Disinfectants Act 1972 (Act 54 of 1972).

***At the time of printing the authors we aware that R918 was replaced by R962 however it was notchanged in the current document because publications were already send using the old regulation. This replacement will thus be made in future documents.**

kind of visit. Unfortunately, South African EHP's are not trained properly on microbial analysis as they rely on visual inspections and there is no use of onsite quick and/or instant analysis instruments to detect possible contaminants. It takes a long time to visit and/or close non-conforming butcheries due to lengthy processes to get evidence to close the place should a need arise (City of Johannesburg, 2014).

Butcheries are required to comply with the regulations contained in the Health Act, 1977 (Act no. 63 of 1977), according to which butcheries are classified as "food premises". In particular, "food premises" must comply with the regulations as set out in the Government Notice as R.918 "Regulations Governing General Hygiene Requirements for Food Premises and the Transport of Food". The R.918 details the requirements for food premises and the duties of employers and owners, but perhaps most significantly, it requires that "no person shall handle food or permit food to be handled (a) on food premises in respect of which a valid certificate of acceptability has not been issued or is not in force", and "(b) in contravention of any restriction or condition or stipulation contained in such certificate of acceptability" (The Butcher, 2014).

However, some butcheries function without being registered by local authorities as it was noted during the selection of some study areas in this current project. Once there is an application for a butchery, an inspector will carry out an inspection, and if he/she is satisfied that the food premises comply with the provisions of the regulations, a certificate of acceptability will be issued in the name of the person in charge (The Butcher, 2014), all these will be based on visual inspections.

R.918 goes into considerable detail regarding the requirements for the food premises, including the surfaces of the walls, ceilings, roofs and floors; wash up facilities; pest control; refuse and waste water disposal; the number and location of latrines, urinal stalls and hand washbasins; areas adjacent to the food handling areas; the working surfaces, tools, utensils and equipment; containers and packaging; chilling, freezing and heating; and protective clothing (The Butcher, 2014). All the mentioned items must be available in working good conditions.

The duties of the person in charge, as well as the duties of the individual food handlers are also set out, as well as requirements relating to the transportation of food and the handling of unprocessed foods. The above places the responsibility on the butcher to ensure only meat derived in accordance with the Meat Safety Act is handled in the butchery. There are several voluntary standards that address the legal requirements. The adoption of these standards will depend to a large extent on the customers' requirements and the major retailers all have their own standards which are audited by each retailer or their representative auditing company. There are a number of South African National Standards (SANS) which are also voluntary, such as SABS 049:2012 for food hygiene requirements and SANS 10330:2007 for a HACCP system. A butcher can choose to implement these requirements and apply for a third party audit by a company such as the SABS (South African Bureau of Standards). This is contrary to what the National Department of Health enforces about HACCP in food processing environments (The Butcher, 2014).

1.5 Consumer knowledge

Meat in South Africa is frequently eaten as part of the consumer's daily diet (Nielsen, 2001). According to Grunert (2006), food (including meat) that is safe, wholesome, processed through acceptable methods and of good eating quality is what the consumer requires in both traditional and retail markets. It is of utmost value to understand the consumers' perceptions and association with the meat product for the industry to remain competitive in the market (Dalle, 2002; Verbeke *et al*., 2010). A study conducted by Vermeulen and Bièbabe (2010) indicated that the main quality attributes and selection criteria for red meat and other products were similar. These includes expiry date, appearance and quality indication, and price, as well as the fat content.

Although there is a resemblance to the studies conducted internationally, the results come with limitations in view of the fact that in Europe, foodborne disease occurrences are reportable. Aspects such as health and environmental concerns, origin and animal welfare including purchase location are of principal significance (Verbeke and Ward, 2006). In terms of chicken meat, attributes such as texture and appearance are indicated as important. Additionally, quality guarantee and expiry date are demonstrated (Vukasovic, 2009). However, internationally, in contrast to South Africa, brand, origin, and packaging are important for selection of chicken and other meat products.

1.6 Rationale

Quality and safety of meat remains an integral part of the food chain and subsequent cross contamination of meat can lead to foodborne illnesses with fatal consequences to the infants, elderly and immune compromised consumers (Sofos, 2008). Foodborne pathogens may remain in the meat cutting equipment, utensils and surfaces in the butcheries thus posing a risk of cross-contamination from one processing day to the next. Moreover, possible cross contamination may occur if the cutting machine and other utensils are used for various meat types without proper cleaning and sanitation (Nørrung and Buncic, 2008; Ali *et al*., 2010). Lack of hygiene during processing, meat cutting and transportation as well as the breach of cold chain could lead to loss of quality through food contamination (Sudhakar *et al*., 2009).

The R.918 details the requirements for food premises and the duties of employers and owners, it further requires that “no person shall handle food or permit food to be handled (a) on food premises in respect of which a valid certificate of acceptability has not been issued or is not in force”, and “(b) in contravention of any restriction or condition or stipulation contained in such certificate of acceptability” (The Butcher, 2014). However, attention must be given to the elimination of pathogenic microbiota from meat in as this is still an ongoing challenge within the meat industry. Although this has led to the implementation of systems such as Good Agricultural Practice (GAPs) and Good Manufacturing Practice (GMPs) during production and processing amongst others; a need is still there to follow on the processes and meat handlers actions during processing.

Concomitant to the above, the Meat Safety Act (South Africa, Department of Agriculture, 2000) 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. Unfortunately, butcheries are only highlighted in R918 as once the meat leaves the abattoir it's within other regulations. In general, regulatory authorities have sought improvement of the microbiological safety of meat by requiring the implementation of Hazard Analysis Critical Control Point (HACCP) systems in all meat packaging and processing 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).

1.6.1 Overall aim

In addition to the rationale above, the challenge faced by the local authorities from non-complying butcheries lead to the initiation of the current study. Moreover, the lack of literature around airborne microbiota in the butcheries as well as the link between workers actions and contaminations in butcheries is lacking. It was therefore the overall aim of this study to asses possible sources of contamination linked with meat handlers

within butcheries as per non-conformance of some butcheries within the selected metropolitan (Mangaung Metropolitan).

1.6.2 Objectives

To obtain the overarching aim of the study, the following objectives were considered:

- To quantify and identify microbial hazards associated with selected butcheries in the Mangaung Metropolitan Municipality.
- To quantify and identify the microbial hazards associated with surfaces and utensils (equipment) as well as their cleanness levels using ATP Hygiena.
- To identify possible microbial hazards associated with food handlers.
- To quantify the airborne microbial population in the meat butchery.
- To assess the level of food handlers knowledge, attitudes and practices regarding safe food handling through a questionnaire.

This project was also intended to shed light to the meat industry especially butchery owners and workers as well as local authority.

1.7 Conclusions

It can be concluded from literature and the rationale that there is a serious need to investigate food handlers' way of conducting their daily work routine and also assess the possible microbial contaminants that could affect the quality of meat products. Moreover, pathogenic strains are of great concern in the meat industry as it has been noticed through a number of projects conducted in South Africa around abattoirs and

meat industry in general. 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.

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Chapter 2

Hygiene practices of meat handlers in butcheries in the Mangaung Metropolitan Municipal area

HYGIENE PRACTICES OF MEAT HANDLERS IN BUTCHERIES IN THE MANGAUNG METROPOLITAN MUNICIPAL AREA

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2.1 Abstract

South Africa is currently amongst countries with a high Human immunodeficiency virus (HIV) prevalence rate (amongst other diseases) and proper food handling is of critical importance for immune-compromised individuals, as well as for children and elderly people. Five butcheries were selected from the Mangaung Metropolitan Municipality due to non-conformance from some of them, in an attempt to assess plant facilities and the personal hygiene of food handlers. A structured questionnaire and a sanitation checklist (observation) were used for the investigation. The structured questionnaire was a self-administered questionnaire which comprised of twelve distinct sections. For each question there were three possible answers (compliant, non-compliant and not sure), intended to obtain the relevant information from the respondents. On average, 70% of the respondents were adhering to good personal hygiene practices, 81% complied with cleaning procedures and 66% were not compliant regarding proper transportation processes. Although the respondents indicated that most proper procedures were followed, in some instances they were not adhered to. This requires further investigation and proper refresher training for employees to ascertain their compliance on issues of good manufacturing practices.

Key words: *Butchery, sanitation, food safety, hygiene, meat handlers, hygiene practice*

2.2 Introduction

For at least a decade, human handling errors in various stages of food service have been known to compound foodborne disease outbreaks (Lues and Van Tonder, 2007; Jevšnik *et al.,* 2008). To date there is no indication that the transmission of microorganisms from food-handlers to food and customers is diminishing, particularly in South Africa (Greig *et al.,* 2007, South Africa, Department of Health, 2007). Moreover, there is a shortage of pathological reporting and a lack of surveillance data although there have been several studies indicating the possibility of contamination of meat due to poor hygiene practices. For instance, literature reports that most foodborne disease outbreaks occur as a result of poor personal hygiene (Nel *et al*., 2004).

In addition, Howes *et al*. (1996) and Clayton *et al*. (2002), reported that 97% of all foodborne illness associated with food preparation areas may originate from workers mishandling food. Unskilled managers and lack of management commitment often add barriers that rob employees of their right to safe food handling (Mason, 2009). On the other hand, the responsibility to provide safe and wholesome meat and to reduce foodborne illness outbreaks also lies with relevant public health authorities (Herenda *et al*., 2000). However, it is crucial to ensure that food handlers are properly trained and well informed about food hygiene and production practices. In recent years, meat safety and quality problems have endangered consumers' health resulting in a negative economic impact of meat production and sales globally (Public Health Agency of Canada, 2008; Nørrung and Buncic, 2008: Pennington, 2009).

Meat cutting is known to be a labour intensive working method and food handler training coupled with education, knowledge, attitude and practices are of paramount importance in managing possible food safety risks (Jay, 1996; Gomes-Neves *et al*., 2011). Butcheries, as an example, are generally regarded as the food handling establishments and they are expected to comply with relevant South African legislation such as the Health Act of 1977, Meat Safety Act of 2000 and the National Health Act of 2003. In addition, butcheries have to consider the implementation of relevant international standards to assist in curbing possible contamination of the final products [South African National Standard (SANS) 10049, 2012].

Knowledge acquired from food safety training on the consequences of processing unsafe food alone has proven to be insufficient in most instances (Griffith, 2000; Angelillo *et al*., 2001; and Askarian *et al*., 2004). The present study was undertaken to determine meat handlers' hygiene practices within butchery premises. Additionally, the study presents assessed data of meat handlers' knowledge, attitude, practices and behaviour (KAPB). This study also sheds light on how meat handlers behave during meat processing and further adds knowledge to the meat industry as there are few reports on butchery hygiene in South Africa in particular.

2.3 Materials and methods

2.3.1 Participating butcheries

Five butcheries (15% of all registered butcheries) with some that have been under scrutiny by the local authorities [Mangaung Metropolitan Municipality, (MMM)] were selected for the purpose of this study. Three of these butcheries were chosen because of their failure to comply with food safety regulations (as informed by MMM) and also because of several reports by the public of non-compliance on hygiene aspects. Two extra butcheries were also used as a control group and for purposes of piloting the checklist and questionnaire.

2.3.2 Questionnaire administration and data collection

A structured self-administered questionnaire for food handlers was used in the study which comprised of twelve distinct sections, with a choice of three possible answers to questions (compliant, non-compliant and not sure) to obtain the relevant information on safe food handling aspects and personal hygiene from the respondents. The interview was structured to reduce response bias and to explain questions which were not clear to the respondents by using open ended questions but directed by the interviewer. Observational study was also conducted in the form of a checklist during the study. Field work was carried out by a team of four members from the Central University of Technology's research unit (Unit of Applied Food Science and -Biotechnology). The team comprised of registered Environmental Health Practitioners (EHP's) who were provided with additional background knowledge on food safety-related aspects. It was

further explained that participation was voluntary and that anonymity would be ensured in the final report. All questions were designed in English but translated into the language the respondents were comfortable with, which where Sesotho, Xhosa, Setswana and Afrikaans which are dominant languages in the Free State province. The questionnaire was administered verbally in the butcheries to a total of 59 respondents covering all food handlers of the selected butcheries.

2.3.3 Data analysis of completed questionnaires and ethical clearance

Upon completion of the interviews the questionnaires were coded and statistically evaluated using Excel 2010 and Sigma Plot 8.0. For the purpose of this project, no ethical clearance was required as by the time the study was conducted there was no need for one until recently when one has to apply for one when dealing with people. Lastly, mangers were also interviewed but the data is not reflected in this report due to sensitivity of the responses and possible danger posed to the workers; however a best amicable solution for all parties was used at the end of the day.

2.4 Results and discussions

The data presented in this chapter has been combined and not separated per butchery due to the similarity of challenges and responses. The results did not suggest any major differences between butcheries hence they were reflected below as combined. This will

assist butcheries, meat industry in general, local authorities in general to be able to cover all aspects, even the one which were not necessarily from respective place.

The first nine questions of the questionnaire focused on the demographic attributes of the respondents. Of a total of 59 meat handlers who participated in this study, 50.8% of the respondents' ages ranged between 22 and 30, with 47.4% being older than 40 years. Twelve percent (12%) of the respondents had no educational background, while a large percentage (45.7%), had a high school educational qualification. None of the respondents had received any form of higher education or food safety training. Forty-nine point two percent (49.2%) of the respondents had worked for more than a year and 40.6% had experience of between 3 and 12 months; only 10.2% had worked for less than 3 months. Fifty-seven percent (57%) of the respondents were permanent staff whilst the rest (42.3%), were contracted and/or seasonal staff. The majority (88.2%) of the workers were African and 11.8% were coloured. The respondents indicated that they would prefer training in their own home language as per the following: 59% (Sesotho), 16.9% (English), 13.55% (Xhosa), 8.4% (Zulu) and 1.7% (Afrikaans). Zulu language was exceptional as this is one of the language less spoken in the Free State province.

The above aspects are crucial as education background, level and home language can be a barrier to employees in understanding both basic and advanced issues of food safety. This may also be added by cultural differences as with the case of some

respondent requesting Zulu in their training. At the same time, there is a tendency among long service employees to relax, and such employees seldom follow the proper food handling procedures due to complacency. Refresher training every six months is therefore crucial especially using languages spoken in the province with a possibility of other languages of the country where necessary such as Zulu language in this case.

2.4.1 Personal hygiene

In terms of the food handlers' role on food safety journey, personal hygiene is a pivotal issue in the maintenance of good health and safety aspects. Several studies have indicated that food handlers are the natural carriers of various microorganisms on their hands, hair and skin, in wounds and in the respiratory tract, as well as on their clothing (Kaferstein, 2003; Bas *et al*., 2006; Ansari-Lari *et al*., 2010). Hand-washing may sound simple and obvious, and this was attested to 86.6% of participants in this study who were aware of how, how often as well as when hands should be washed (Table 2.1). However, during observation, it was clear that respondents were not practising what they knew possibly as a result of lack of hand-washing facilities and time. These challenges can be supported by recent hand-washing campaign that have been presented in South Africa since 2010 to date by the department of Health and Water Affairs (personal observation). Additionally, respondents reported that unreasonable demands made by management such as reaching a certain target per day, particularly at the end of each month, added to the failure to adhere to proper hygiene practices. This challenge is visible in the metropolitan during pick days such as month ends,

Table 2.1: Assessment of meat handlers' personal hygiene knowledge, attitude and practices including behaviour in five butcheries

Personal hygiene items	**% Compliant**	**% Non-compliant**	**% Not sure**
Meat should be handled by persons with clean hands, fingernails and clothes	80.0	20.0	0.0
Hands should be washed thoroughly with soap after every visit to a latrine or toilet	88.6	11.3	0.0
Wounds, cuts and sores should be covered	45.7	45.7	8.5
Transmission of microorganisms from man to food occur through a carrier	85.3	14.7	0.0
It is not important to wash hands frequently when you wear gloves	21.3	30.2	48.5
Food handlers can wear jewellery in meat plant	50	44.1	5.8
At least 20 seconds is enough for proper hand washing	26.2	50.7	23.1
Standard Deviation (STD)	28.1	16.1	18.0
Group data standard deviation (STD_g)	27.6		

Christmas holidays, and Easter holidays and during major activities such as Mangaung Cultural Festival (Macufe) in this region.

Adding to the further points, 62.7% of meat handlers had little knowledge of policy relating to the wearing of jewellery, and 90% of workers were wearing jewellery during meat processing. It is clearly stated in Regulation R.918 informed by the Health Act of 1977 and other related legislation (South Africa, Department of Health, 1977; South Africa, Department of Health, 2000), that it is not acceptable to wear wrist watches, earrings, bracelets or rings as they pose a risk of physical contamination as they can harbour bacteria. Except for a plain ring such as a wedding band, it is unacceptable for food handlers to wear jewellery including the medical jewellery on their hands and arms during food processing. Although 60% of butchery employees did not have protective clothing, 80% of the respondents acknowledged that suitable clean protective clothing must be worn to prevent contamination of food from regular clothing (Table 2.1).

2.4.2 Protective clothing

During operational activities, pathogens have the potential to contaminate clothing through dirty material. Meat contact surfaces such as utensils and equipment become contaminated as a result of workers moving around in the meat processing areas with contaminated clothing (Forsythe and Hayes, 1998). It is a legal requirement that meat handlers be provided with clean and light-coloured protective clothing, including head covering, beard nets and footwear to prevent meat from being contaminated (South

Africa, 1999). Eighty-six percent (86%) of the respondents reported that they always take their protective clothing home to be washed when it becomes soiled (Table 2.2). Dressing employees properly for the meat processing plant will not only protect them from unnecessary injuries, but will also give a good image and send a positive message to the consumer about the level of sanitation in the establishment.

2.4.3 Training

Education and training on food safety programmes are fundamental tools that should be utilised to help meat handlers understand what is expected of them and the reason why it is important to ensure a food safety culture (Egan *et al*., 2007). All of the participants (100%) indicated that they had never followed the training course regarding food safety whilst managers indicated that they had not received formal training instead they had received informal training in the form of worker to worker training. This one on one training did not include food safety aspects, however (Table 2.3) shows that not all employees were trained. To substantiate this statement, it is important to note that the participants thought that the questionnaires were a form of formal food safety training and requested certificates afterwards. Ninety-six percent (96%) of the respondents in this study showed an acceptable attitude towards food safety training, although such training may not necessarily result in significant behavioural changes (Ansari-Lari *et al*., 2010).

Table 2.2: Assessment of meat handlers' protective clothing knowledge, attitude and practices including behaviour in five butcheries

Protective clothing	**% Compliant**	**% Non-compliant**	**% Not sure**
Washing and cleansing of steel mesh gloves should occur at regular intervals	93.0	0.0	7.0
Frequently cleaning gumboots	93.0	2.0	5.0
Protective clothing washed at home	86.0	14.0	0.0
Wearing of clean protective clothing at the start of each shift	83.0	10.0	7.0
Protective clothing worn by everyone entering processing area	84.0	16.0	0.0
Wearing of hairnets, beard nets during meat handling	93.0	7.0	0.0
Standard Deviation (STD)	24.0	17.1	8.1
Group data standard deviation (STD_g)	37.7		

Table 2.3: Assessment of meat handler's food safety training in five butcheries

Food safety training	**% Compliant**	**% Non-compliant**	**% Not sure**
Provision of training to all the workers	100	0.0	0.0
Appropriate skills and knowledge in food hygiene	96.0	3.0	1.0
Standard Deviation (STD)	2.8	2.1	0.7
Group data standard deviation (STD_g)	50.1		

2.4.4 Transport

Failure to adhere to the legal requirements of transportation of meat can slightly increase the level of contamination, leading to deterioration of meat quality with inadequate shelf life. The safety of meat and meat products can be compromised and may possibly create a serious health risk, if the cold chain is broken. This can under no circumstances be rectified. The results of the present study showed lack of knowledge in this regard, and relatively poor practices, as 66.1% of the respondents indicated that people can be simultaneously transported with the meat (Table 2.4). Consequently, the statement contradicts Regulation R918 which stipulates the hygiene requirements for transportation of food, promulgated under the Health Act, Act no. 61 of 2003 (South Africa, Department of Health, 2003), stating that a vehicle used for transportation of butcher's meat shall not be used concurrently for the transportation of any item or person who will sit or stand on carcasses, thus contaminating the meat.

2.4.5 Storage

Meat quality and safety are frequently compromised as a result of improper storage (Education Foundation, 2004). Approximately sixty-eight percent (67.8%) of the respondents incorrectly answered the question related to separation of raw meat and ready-to-eat meat during storage (Table 2.5). Comparing the results with similar studies conducted in Portugal (Gomes-Neves *et al.,* 2007), the level of knowledge was found to be acceptable. In recent years, outbreaks of *Listeria* poisoning have been linked to

Table 2.4: Assessment of meat handlers' transportation knowledge, attitude and practices including behaviour in five butcheries

Transportation	**% Compliant**	**% Non-compliant**	**% Not sure**
Cold chain maintenance	100	0.0	0.0
Inspection of transport	93.2	3.4	3.9
Vehicle transporting meat can carry people	32.2	66.1	1.7
Standard Deviation (STD)	37.3	37.2	2.0
Group data standard deviation (STD_g)	42.0		

Table 2.5: Assessment of meat handlers' storage knowledge, attitude and practices including behaviour in five butcheries

Storage	**% Compliant**	**% Non-compliant**	**% Not sure**
Meat storage room should be clean and sufficient	96.6	3.4	0.0
Storage of meat to prevent cross contamination from different meat species	86.4	13.6	0.0
Raw meat cannot be stored with ready-to-eat meat	32.2	67.8	0.0
Meat labelling important	32.2	67.8	0.0
One way to rotate meat products is to follow first-in, first-out (FIFO)	88.1	10.2	0.0
Never store food with chemicals	28.8	71.2	0.0
Store at a min of 15 cm away from the wall and 15 cm away from the floor	91.5	8.5	0.0
Standard Deviation (STD)	32.0	32.2	0
Group data standard deviation (STD_g)	37.0		

contamination of ready-to-eat (RTE) and raw meat (Nørrung and Buncic, 2008) emphasising the need to separate various species and proper storage of meat products.

2.4.6 Receipt of goods

In order to ensure that the meat served is safe and wholesome, meat establishments should have designated areas for deliveries to reduce the possibility of encountering potential meat safety hazards. In this study, only 40% of the butcheries had designated areas suitable to control the raw materials (e.g. frozen, refrigerated and chilled carcasses) upon arrival and protecting the unloaded meat from any alterations that may be caused by the external environment. Fifty-one percent (51%) of the respondents stated that this specification was not important when receiving the meat. The majority of the respondents (83%) reported that they always check the stamp of conformity so as to ensure that the meat carcasses had undergone mandatory inspection enforced by the South African Department of Agriculture.

Seventy-eight percent (78%) of the participants agreed that checking the temperature of the vehicle and the meat was important but, (10.2%) disagreed with the statement while 11.8% did not know the answer (Table 2.6). However, only 20% of the butcheries had an in-house thermometer (observational studies) which proved in the current study that although the food handlers have good knowledge towards food safety they do not always put the knowledge into practice. Numerous studies demonstrated that inadequate temperature control of food is the main cause of food poisoning and food

Table 2.6: Assessment of meat handlers' receiving of raw material (meat) knowledge, attitude and practices including behaviour in five butcheries

Receiving	**% Compliant**	**% Non-compliant**	**% Not sure**
Specification important	45.8	50.85	3.4
Inspection stamps indicate licensed abattoir	83.1	16.95	0.00
Inspection of vehicle for temperature important.	78.0	10.2	11.8
Meat received always free of contamination	89.8	6.8	3.4
Frozen meat upon arrival must be stored at -18ºC in 10 min	66.1	13.6	20.3
Standard Deviation (STD)	17.3	17.8	8.2
Group data standard deviation (STD_g)	32.3		

handlers lack this knowledge as a measure to prevent and reduce the risk of foodborne pathogen growth to an infectious level (Walker *et al*., 2003; McCabe-Sellers and Beattie, 2004; Bas *et al*., 2006; Gomes-Neves *et al.,* 2007; Jevšnik *et al*., 2008)

2.4.7 Temperatures

The regulations of the National Health Act (Act no. 61 of 2003) clearly stipulate that it is the owner of the butchery's responsibility to ensure that the meat handlers understand that the maintenance of correct temperature and the prevention of contamination is imperative to comply with food safety regulations (South Africa, Department of Health, 2003). Table 2.7 shows that approximately 28% of the meat handlers indicated that they were not sure about whether frozen meat may or may not be dispatched at core temperature higher than -12ºC, whilst 11.8% did not comply in this aspect. The red meat regulations contained in the Meat Safety Act (Act no. 40 of 2000) (South Africa, 2004) clearly stipulate the requirements related to the above. Over and above regulations, cost reduction by minimizing wastage may be ensured by the maintenance of the correct temperature. Sixty-six percent (66%) of respondents' knowledge and practices with regard to rotating refrigerated and frozen products to ensure that the meat products with the earliest use-by or expiry dates are identified first were unacceptable.

Table 2.7: Assessment of meat handlers' temperature knowledge, attitude and practices including behaviour in five butcheries

Temperatures	**% Compliant**	**% Non-compliant**	**% Not sure**
Refrigeration slows microbial growth	91.5	3.4	5.1
Spoilage due to bone taint is unlikely to occur at 7°C	72.9	15.3	11.8
Refrigerators are cleaned weekly	77.9	16.9	5.2
Verification of the internal meat temperature is done with a use of a thermometer	76.3	6.8	16.9
Maximum temperature for the dispatch of frozen meat is -12°C	59.3	11.9	28.8
Cold rooms arranged in a first-in first-out basis	66.1	27.1	6.8
Standard Deviation (STD)	11.0	8.3	9.2
Group data standard deviation (STD_g)	31.0		

2.4.8 Equipment and utensils

In general the design of the equipment used in the butcheries for processing meat is such that equipment is not easy for employees to clean and sanitize, especially without the equipment manufacturer's cleaning instructions. Forty-six percent (46%) of respondents seem to be practising poor cleaning procedures and sanitation of the equipment, according to their responses to the question "the hard to dissemble machine can be left out for inspection and the clean-up", about 13.5% indicated that they were not sure as they had never seen anyone inspecting the machines. Eighty-eight percent (88%) of the respondents appeared to be following the correct steps (pre-cleaning, cleaning, rinsing, disinfection, rinsing and drying) in regard to equipment washing and disinfection, while 8.4% stated that they do not follow the steps, and 3.4% were not sure of the correct procedure (Table 2.8).

2.4.9 Foodborne pathogens

Major safety concerns for the meat industry are pathogenic microorganisms (Bhandare *et al.*, 2007; Sofos, 2008). Although foodborne pathogens are not generally detectable by the unaided eye, they may contaminate meat contact surfaces in various ways from humans, insects, air and water. Even though only 20% of the butcheries kept records and standard operating procedures (SOP), 90% of the respondents agreed with the statement that "90% or more of bacteria can be removed by detailed SOP in place". About 6.7% did not agree with the statement and 3.4% were not sure about SOP method. Fifty-eight percent (58%) of the respondents did not know that physical

Table 2.8: Assessment of meat handlers' equipment knowledge, attitude and practices including behaviour in five butcheries

Equipment	**% Compliant**	**% Non-compliant**	**% Not sure**
Build-up of meat residues on meat cutting equipment can serve as a breeding place for insects and bacteria	91.5	8.5	0.00
To prevent contamination all meat contact surfaces should be sanitised as often as necessary	91.5	3.4	5.1
The hard to dissemble machine can be left out for inspection and the cleaning	40.7	45.8	13.5
Contamination of surfaces/products can occur due to build-up of or seepage of cleaning solvents.	42.4	47.5	10.1
Dead spaces in and around equipment can collect bacteria or insects	84.8	10.1	5.1
Equipment must be cleaned, rinsed, sanitized and allowed to air dry	88.1	8.5	3.4
Standard Deviation (STD)	24.6	20.3	4.8
Group data standard deviation (STD_g)	34.4		

cleaning with the use of high pressure hoses can produce contaminated water droplets (aerosols) which could taint the meat (results indicated in Table 2.9).

2.4.10 Plant sanitation

The primary objective of keeping the meat plant in a sanitary condition is to prevent the production of unattractive, tasteless products and also to control the microorganisms in order to reduce the health hazards that might be present (Quintavalla, 2010). In this study lack of knowledge of plant sanitation was revealed as 62.7% of the respondents indicated that removing garbage frequently to maintain meat premises in a sanitary condition was inadequate (Table 2.10). Proper garbage handling can reduce the problems related to odour and pests.

2.4.11 Effective cleaning

Keeping the meat plant in a clean and sanitary condition is a common safe practice since this attracts customers. The main concerns are however, the activities that involve the mincing and cutting, and the sausage and patty making. The knowledge of the present study population regarding the general sanitary measures such as four basic steps of washing hands and effective cleaning of the equipment in the meat processing areas was generally poor. Approximately eighty-five percent (84.8%) of the respondents agreed that there is a huge difference between cleaning and sanitizing, as indicated in Table 2.11. However, only 10% of the butcheries used the sanitizing solutions, which indicates that in 90% of the butcheries a very high risk of meat contamination is posed

Table 2.9: Assessment of meat handlers' foodborne pathogen knowledge, attitude and practices including behaviour in five butcheries

Foodborne pathogens	**% Compliant**	**% Non-compliant**	**% Not sure**
90% of bacteria can be removed by standard operating procedures (SOPs) in place	89.8	6.8	3.4
Bacteria can be present on a sparkling clean surface	37.3	62.7	0.00
Plant sanitation should be audited by an outside source such as a cleaning product supplier	66.1	22.0	11.9
Cracked walls, floor and ceiling may harbour bacteria	94.9	3.4	1.7
Some foodborne pathogens can survive in dry conditions	93.2	3.4	3.4
Microorganisms cannot travel throughout the plant in water droplets generated by the use high pressure hoses	57.6	30.5	11.9
Even healthy persons can harbour microorganisms in their (nose, hands, fingernails and on their skin)	91.5	5.1	3.4
Standard Deviation (STD)	22.4	21.9	4.8
Group data standard deviation (STD_g)	35.8		

Table 2.10: Assessment of meat handlers' meat plant sanitation knowledge, attitude and practices in five butcheries

Plant sanitation	**% Compliant**	**% Non-compliant**	**% Not sure**
Keeping the processing surfaces clean can reduce public health risks	89.8	6.8	3.4
Clean walls and floors can only be identified visually	69.5	30.5	0.00
Adequate lighting and ventilation should be provided throughout the facility	94.9	3.4	1.7
Frequent removal of garbage is essential	62.7	37.3	0.00
Colour coded brushes should be used	93.2	1.7	5.1
Standard Deviation (STD)	14.8	16.7	2.2
Group data standard deviation (STD_g)	38.1		

Table 2.11: Assessment of meat handlers' efficient cleaning knowledge, attitude and practices in five butcheries

Efficient cleaning	**% Compliant**	**% Non-compliant**	**% Not sure**
Cleaning schedule include detailed instructions for cleaning all areas of the facility	78.0	17.0	5.0
A cleaning schedule should be used all the time	81.4	11.9	6.8
Chemical manufacturer's instructions should always be followed during cleaning	86.4	8.5	5.1
Chemicals should be stored in a locked area	44.1	55.9	0.0
There's a difference between cleaning and sanitizing	84.8	11.9	3.3
Effectiveness of sanitizer is determined by the right proportion of (H_2O: sanitizer)	98.3	0.00	1.7
Temperature of water important during cleaning	84.8	10.2	5.0
Read instructions before using chemicals	93.2	1.7	5.1
Standard Deviation (STD)	16.4	17.6	2.2
Group data standard deviation (STD_g)	37.4		

as microbes can persist on surfaces that have only been cleaned and resulting in biofilm formation. The great majority, 98.3%, agreed with the statement “it is important to follow manufacturer’s instructions for cleaning”, except in addition to their answer they further indicated that they do not have a cleaning procedure or schedule in place and viewed the exercise to be a waste of time as they rely mainly on experience acquired. According to Heinz and Hautzinger (2007), in a meat plant the cleaning and sanitation procedures are of the utmost importance. However, the procedure is habitually neglected since it takes extra time and hard work to remove organic matter such as fats and protein particles from surfaces (walls, floors and equipment).

2.4.12 Pest control

Pests (rats, mice, cockroaches, flies and ants, as well as birds and insects) in food processing premises have long been recognised as food safety hazards and a risk or threat to the health of the consumers. These hazards are potentially introduced by poor hygiene practices. Moreover, not only are they undesirable hazards because they can contaminate food with foreign bodies such as faeces and hair, but they may also be carriers of deadly disease (Howard, 1999; SANS 10049, 2012). In the present study, lack of knowledge, attitude and practices was observed with regard to pest control, as 57.6% respondents indicated that they normally experience evidence of damage and debris caused by rodents and insects. From the five butcheries, only two evidenced adequate control of pests. Although records were not available, it was possible to identify the pest control devices correctly (Table 2.12).

Table 2.12: Assessment of meat handlers' pest control knowledge, attitude and practices in five butcheries

Pest control	**% Compliant**	**% Non-compliant**	**% Not sure**
Human hazard and precautionary statement appears on a label of pest control devices	64.4	11.9	23.7
The devices are easily identifiable	59.3	13.6	27.1
Rodents bait station tamper-resistant and secured to the ground	55.9	18.6	25.5
Fly lights properly positioned	66.0	13.6	20.4
Do you normally experience evidence of damage and debris caused by insects	57.6	27.1	15.3
Standard Deviation (STD)	4.4	6.2	4.7
Group data standard deviation (STD_g)	20.7		

2.5 Conclusion

The study reflects an overview of the personal and general hygiene practices of food handlers in butcheries and it may be concluded that most butcheries and their employees were aware of the required procedures. However, not all complied with the required procedures even though they were aware of their implications. Refresher training and total adherence must be mandatory by all food handlers in general. It will be best to have refresher training courses every six months provided by butchery management through consultation with private consultants and/or local authorities. Environmental Health Practitioners should include training as part of their hygiene inspection visits to butcheries. Such training could prohibit the ineffectual employee to employee training which has been reported to be the case during the study. The challenges could include a language barrier as people preferred using their own language for training purposes. The maintenance of and improvements to the infrastructural butchery facilities henceforth should be the subject of investment agenda.

The Mangaung Metropolitan Municipality (MMM), ought to guide and advice that all the meat establishments be installed with easy to clean equipment and that they are equipped with adequate hand-washing facilities and a rest room. This includes a programme of inspections and audits by the local authorities to stimulate actions which can attend to existing problems with immediate effect. However, MMM can also suggests private inspectors and auditors within their by-laws to help butcheries to attain good and acceptable working standards. The most prevalent barriers to safe meat-

handling practices indicated by workers were lack of time, unhygienic designs and insufficient resources. For owners to remain on the cutting edge of the technological advances in cleanliness of the butcheries, they should engage the services of professional hygiene and food safety professionals. Understanding the reasons why food hygiene practices are important would be likely to result in more healthy behaviour.

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Chapter 3

Fingerprinting of bioaerosols in butcheries using MALDI-TOF MS

FINGERPRINTING OF BIOAEROSOLS IN BUTCHERIES USING MALDI-TOF MS

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3.1 Abstract

Airborne contaminants are one the most pressing concerns in the meat processing industry and have been recently documented to result in a negative impact on the safety and wholesomeness of meat products, as well as on the health of employees in the meat industry. This study aimed at characterizing and ascertaining the levels of airborne contaminants in butcheries in the Mangaung Metropolitan municipal area. Air samples were impacted on agar using SAS–Super 90 and quantified. Subsequent identification and fingerprinting was done using Matrix Assisted Laser Desorption Time of Flight - Mass Spectrometry (MALDI-TOF MS). The total levels of airborne contaminants over the entire duration of the study ranged between 2.7×10^2 and 5.41×10^3 $cfu.m^{-3}$ at an average of between 5.4×10^1 and 1.08×10^3 $cfu.m^{-3}$. Fingerprinted airborne contaminants included strains that are commonly known for spoilage of meat, faecal contaminants and nosocomial infection pathogens. The dominant isolated genera included *Bacillus, Kocuria, E. coli*, *Neisseria, Staphylococcus, Campylobacter* and *Pseudomonas* amongst others. The prevalence of these airborne contaminants at butcheries not only hinted at the possible health hazards to consumers but also highlighted the status of airborne contamination with a possibility to adversely affect the safety and quality of meat products, with possible negative health effects. A need for improved ventilation at butcheries was also observed so as to control the prevalence and distribution of these airborne contaminants. The findings of this study emphasized a need for agreed standards in relation to levels of airborne contaminants.

Key words: *butcheries, airborne contaminants, bioaerosols, food safety*

3.2 Introduction

Bioaerosols are defined as airborne particles originating from biological sources including bacteria, fungi and viruses (Lutgring *et al*., 1997; Srikanth *et al*., 2008). They are fast becoming well known as contaminants in the meat, dairy and food processing industries. These bioaerosols are ubiquitous and normally attached to solid particles of dust, clothing, hair or soil, or to liquids such as water and condensation in the air. Bioaerosols are known to cause economic loss and human health problems. However, these microbial airborne contaminants (bioaerosols) have not been given much attention due to the lack of research interest and lack of agreed standards internationally (Lutgring *et al*., 1997; Wirtanen *et al*., 2002; Srikanth *et al*., 2008). Srikanth *et al*. (2008) further reported that the threshold limit of bioaerosols for the assessment of health impacts, dose and toxic effect has not yet been established or agreed upon; however, their presence in the processing environments can cause numerous undesirable health effects.

In most food processing environments such as dairy plants (Kang and Frank, 1989; Ren and Frank, 1992), abattoirs (Knudtson and Hartman, 1993), and poultry plants (Lutgring *et al*., 1997; Theron, 2003; Northcutt *et al*., 2004), the air has long been acknowledged as a possible source of microbial contamination. On the other hand, the health of the food handlers may play a significant role as air can be contaminated when respiratory droplets are dispersed into the air during sneezing, talking, working and breathing (Cundith *et al*., 2002). Furthermore, most of microorganisms can be aerosolized from

carcass blood spills and high pressure spraying of surfaces in the butcheries and abattoirs. Reduction of quality and shelf life of meat products, including sick building-related syndrome and nosocomial infections due to bioaerosols, are some of the challenges faced due to poor ventilation systems in most processing plants (Jay, 2002).

Bioaerosols are known to be most prevalent in poorly maintained building structures where there are cracked walls and floors, improperly sealed doors and inefficient ventilation systems (Jones *et al.,* 2003; Srikanth *et al*., 2008). Infiltration through damaged and corroded roof structures may lead to excess humidity causing condensation on the walls and ceiling, which may contaminate food through dripping. The design parameters governing ventilation should be extended to accommodate adequate intake of fresh air to match the exhausted air to enable consistent flow in the receiving areas (Lutgring *et al*., 1997). Installation of an efficient air conditioning system could place restrictions on the spread and rapid growth of microbial contaminants.

Therefore, the aim of the study was to enumerate total indicator organisms and identify prevalent species in the butchery premises. This study will cast light on the hygiene practices and processes within the studied butcheries and the possible impact(s) of bioaerosols on meat products.

3.3 Materials and methods

3.3.1 Sampling site

Air samples collected in the study were obtained from five butcheries (15% of total registered butcheries) within the Mangaung Metropolitan municipal jurisdictional area. Butcheries A, B and C were located in the central business district whilst butcheries D and E were located outside the business district area, in the township area. Butcheries A, B and C operate for twelve hours a day each, with 21, 17 and 5 employees respectively. Butchery D operates for 12-16 hours a day depending on the number of customers as it also has a barbeque (braai) facility; this butchery has approximately six employees. Butchery E operates for fifteen hours with approximately 26 employees divided over two shifts per day. Working operations at these meat processing facilities are similar (apart from barbecuing at butchery D) and include trimming, mincing and sausage processing, amongst other processes.

3.3.2 Sampling protocol

At least duplicate air samples were obtained from four processing areas per butchery within a period of five months, before and during meat processing. The processing area included: the main entrance, processing areas 1 and 2, as well as the display area. Airborne microbes were collected onto agar surface of 55-mm RODAC plates by impaction at nominal air flow rate of 100 $l.min^{-1}$. The initial counts were total viable count (TVC) using Plate Count Agar, (MERCK, RSA), incubated at 25°C for 48h and expressed as LOG $CFU.g^{-1}$ where necessary (Bryan *et al*., 1996) prior to identification

through MALDI TOF MS. A single stage surface Air Sampler (SAS Super 90) (PBI International, Milan, Italy) was used for this purpose, and fixed onto a tripod stand at a height of 1.5 m (average breathing zone of workers) from the floor. Pre-calibration to a flow rate of 28 $l.min^{-1}$ was done prior to use. At the beginning of each sampling the aspiration head was autoclaved at 121ºC for 15 min and between each sample run 70% ethanol was used for the sterilization of the lid (Theron, 2003). Plate count agar plates were incubated at 25ºC for 48-72 h. Colonies were counted manually, and recorded as colony-forming units per cubic metres ($cfu.m^{-3}$) of air.

3.3.3 Identification of microorganisms

Microflex™ LT benchtop, autoflex™ with BioTyper™, COMPASS™ for FLEX series incl. flexAnalysis™ MALDI-TOF MS (Bruker Daltronics, South Africa) was used for the purpose of taxonomic identification and fingerprinting of isolated airborne microorganisms. Single colonies from biological material were picked up from the prepared Plate Count Agar and transferred into an Eppendorf tube with 300 *µ*l of ultra-pure water (Merck, SA) and homogenized or vortexed. Absolute ethanol (900 *µ*l) was added carefully, vortexed, and centrifuged at maximum speed (13200 x g speed) for approximately 2 min at room temperature. The supernatant was decanted and the pellet air-dried at room temperature. Dry pellets were vortexed with 50 *µ*l formic acid (70%) (Merck, SA), followed by the addition of 50 *µ*l pure acetonitrile (Merck, USA) and further mixed thoroughly. The mixture was centrifuged at maximum (13200 x g speed) for 2 min, and approximately 1 *µl* of the supernatant was placed onto a Micro Scout Plate

(MSP) 96 polished steel target plate (Bruker Daltronics, Germany) and allowed to air dry at room temperature. Consequently, each sample was overlaid with 1 *µl* of the HCCA matrix solution (a saturated solution of a-cyano-4-hydroxy-cinnamic acid (Sigma, USA) in 50% acetonitrile-2.5% trifluoroacetic acid) (Bruker Daltronics, Germany) and air dried at room temperature. The analysis of all strains was performed with Microflex LT (light Transmitter) mass spectrometer (Bruker Daltronics, Germany) using Flex Control software (Version 3.0, Bruker Daltronics, Germany). The spectra were recorded in the linear positive mode (with the laser frequency of 20 Hz; ion source of 1 voltage, 20kV; ion source of 2 voltage, 18.6 kV; lens voltage, 7.5 kV; mass range, 2 to 2 000 kDa). For each spectrum, 240 shots in 40-shotsfrom different positions of the Bacterial Test Standard (BTS) spot (manual mode) were collected and analysed. The spectra were internally calibrated using *Escherichia coli* ribosomal proteins as the standard. The raw spectra were imported into the BioTyper software (Version 3.0, Bruker Daltronics, Germany), processed by standard pattern matching with standard settings, and the results reported in a ranking table with colour codes. Outcomes of the pattern-matching process were expressed as proposed by MALDI-TOF BioTyper (MT) manufacturer with identity scores ranging from 0 to 3. Scores of less than 1.70 were considered not to have generated a reliable identification; a score of between 1.7 and 1.9 was considered to be precise genus identification, with a score above 1.9 considered to be reliable species identification.

3.4 Results and discussion

3.4.1 Total viable counts

The quality of air in the food processing environments (including meat processing environments such as butcheries and abattoirs) plays a key role in the safety and quality of products as microorganisms that cause spoilage are able to spread easily via the air, causing contamination and possible food poisoning (Kang and Frank, 1989; Rahkio and Korkeala, 1997; Whyte *et al*., 2001; Cundith *et al.,* 2002; Sutton, 2004; Shale, 2004). A summary of microbial loads at butcheries in the Mangaung Metropolitan municipal area is presented in Figure 3.1. Overall, the total microorganism concentrations at butcheries ranged from 2.70×10^2 to 5.41×10^3 cfu.m^{-3} with the average concentrations ranging between 1.53×10^2 and 1.08×10^3 cfu.m^{-3} over the entire duration of the study (selected positive correlations coefficients and grouped data standard deviations highlighted below). The total and average microorganism levels were comparable to those of De Koster and Thorne (1995), Pastuszka *et al.* (2000) and Von Tayson (2009), in their respective studies. Microbial counts at butchery A were observed at the lowest and highest levels of 2.30×10^1 cfu.m^{-3} and 6.5×10^1 cfu.m^{-3} respectively. The high level of counts was observed in processing area 1; these could be attributed to activities in that section with the handling of products, movement of employees, saw-dust on the floor used to make the floors less slippery, and the surfaces of the processing equipment (Stetzenbach, 1997; Johannessen *et al.,* 2002). The strong positive correlation recorded in this butchery was between processing area 1 and 2 (r=0.96) followed by (r=0.95) between main entrance and the display sections. The group standard deviation was recorded at STD_g=10.53 for butchery A.

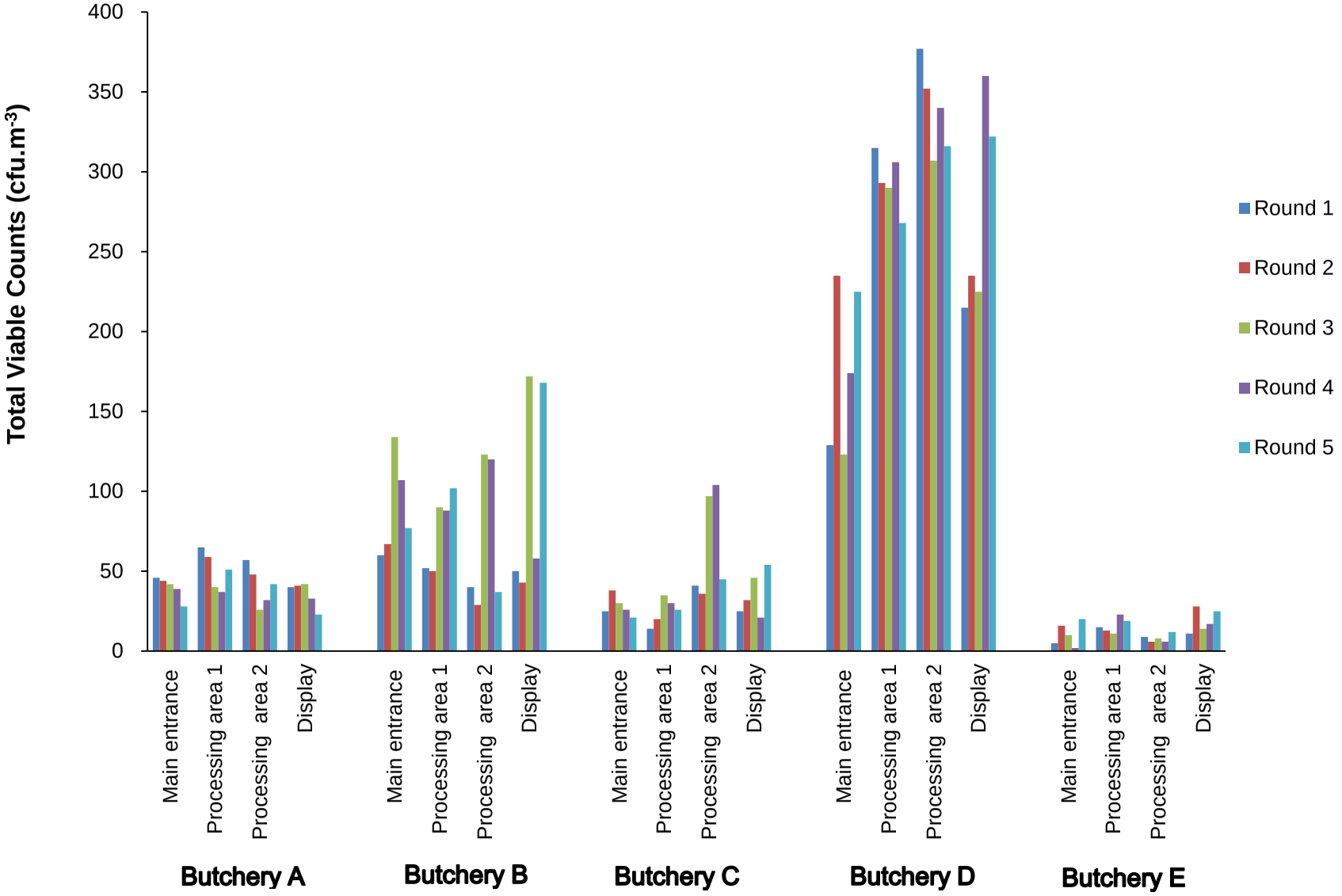

Figure 3.1: Distribution of airborne microbial loads at butcheries

In contrast to butchery A, in butchery B the lowest and highest counts observed were 2.9×10^1 cfu.m^{-3} and 1.72×10^2 cfu.m^{-3}. The high level of microbial counts in the display area may have originated from the open doors (remains open all day long) which allow access to outside air, the fluctuation of ambient air and humidity, the high traffic movement of employees and customers, the display fridge operation and the set up (Rahkio and Korkeala, 1997; Helm-Archer *et al.,* 2004). A moderate positive correlation of (r=0.79) was recorded between processing area 1 and the display section. Butchery C appeared to have low counts of between 1.40×10^1 cfu.m^{-3} and 1.04×10^2 cfu.m^{-3} with STD_g=41.7 and the moderate positive correlation noted between processing areas 1 and 2 (r=0.83).

In this environment, the level of hygiene was good and there were a limited number of employees: this could have contributed to the low levels of bioaerosols (Lehto *et al.,* 2011). The highest counts were found in the processing area 2, and could have emanated from the drainage system in the area that was not covered. This was in agreement with the findings of Salustiano *et al.* (2003), in a study they conducted where high counts were observed in the processing area at a dairy plant. In butchery D, high levels of airborne microbes (ranging between 1.23×10^2 cfu.m^{-3} and 3.77×10^2 cfu.m^{-3}; STD_g=71.4) and the moderate positive correlation of (r=0.75) between processing areas 1 and 2 were found in all the processing areas, presenting a different pattern in comparison with other sampled butcheries. The distribution and the high counts of bioaerosols in all areas of this establishment could be attributed to the setting of the

building and the incorporation of the barbeque in the vicinity of the main entrance which is also used as the receiving area (Macher, 1999; Qudiesat *et al.,* 2009).

Lastly with regard to quantified values, butchery E showed the lowest levels in comparison with the other butcheries, with the lowest level of 6.0×10^{0} cfu.m^{-3} and a high of 2.8×10^{1} cfu.m^{-3} in addition to the moderate positive correlation of (r=0.78) between main entrance and the display section as well as the group standard deviation STD_g=6.8 for butchery E. These low levels of airborne microorganisms at butchery E could be attributed to the design of the building, availability of air conditioning systems and good level of personal and general hygiene found in the meat establishment (Godish, 1995; Kalliokoski, 2003; Lehto *et al.,* 2011). Although the information on the concentration of bioaerosols in butcheries is limited, the counts in the current study were lower than those obtained by Lues *et al*. (2007) and Shale (2004), who obtained high microbial levels in the de-feathering area in a chicken slaughtering facility and the processing areas at abattoirs respectively. In addition, Table 3.1 below reflect significant differences between butcheries over the entire sampling period with regard to airborne total viable counts quantified.

3.4.2 Profile of airborne microbial species at butcheries

This section will report on selected isolates which are related to food safety issues, but all isolated strains are depicted in the respective tables per butchery. Numerous studies of bioaerosols contamination levels in meat processing facilities have indicated airborne

Table: 3.1: Significant values for airborne TVC counts within selected butcheries.

Butcheries grouping	**Significant value (p)**
A and B	1.41×10^{-7}
A and C	0.001
A and D	1.05×10^{-11}
A and E	0.06
B and C	0.01
B and D	0.024
B and E	6.28×10^{-11}
C and D	7.42×10^{-6}
C and E	2.05×10^{-6}
D and E	3.12×10^{-15}

microbiota as the potential source of contamination in meat and meat products (Kotula and Emswiler-Rose, 1988; Rahkio and Korkeala, 1997; Jericho *et al*., 2000). Although a number of studies have been done at meat processing plants (Shale*,* 2004), there is nevertheless only a limited amount of literature on the prevalence and distribution of airborne microbes and the microbiological contamination of meat products at butcheries as most of the studies were conducted at abattoirs. The composition of bioaerosols microbiota in butcheries in the current study included Gram-positive and Gram-negative bacteria as listed in Tables 3.2, 3.3, 3.4, 3.5 and 3.6. These pathogens of concern included *Bacillus, Campylobacter, Lactobacillus, Pseudomonas, Escherichia coli, Hafnia, Staphylococcus, Yersinia and Neisseria* amongst others.

Many Gram-positive species are widely distributed in the environment and are commonly associated with meat, seafood and dairy food contamination (Böhme *et al*., 2011). At butcheries, a variety of microorganisms that are normally associated with the spoilage of meat and implications in foodborne disease outbreaks were isolated, as shown in Tables 3.2-3.6.

The first microbe of concern was *Bacillus* species which have been associated with foodborne disease outbreaks from as early as 1906 with a series of outbreaks in Europe; however, they were not conclusively established to be the cause of food poisoning and foodborne disease outbreaks until 1950. *Bacillus* spp. was the dominant Gram-positive aerobic bacteria isolated from all the sampled areas at different butcheries (A, B, C, D, and E) in the current study. This was not alarming since they are

Table 3.2: Isolated microorganisms from butchery A

Areas	Organism	Isolated from	Implications	References
Main entrance	*Bacillus pumilus DSM 1794 DSM*	Soil and aquatic environments.	Food spoilage organism causing skin infections in immuno-compromised humans. Reduce infection of plant roots by certain fungi, and act as a pesticide. Produces an antibiotic pumilin.	Tena *et al.,* 2007
	Lactococcus garvieae DSM 6783 DSM	Cattle and humans; kidney of diseased yellowtail fish.	Possibility of zoonosis and important pathogen in aquaculture.	Raissy and Ansari**,** 2011
	Micrococcus luteus IMET 11249 HKJ	Soil, dust, water, air, and normal flora of mammalian skin. Also colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007
	Enterococcus faecalis DSM 20409 DSM	Inhabitant of the gastrointestinal tracts of a wide variety of insects and animals, including humans.	Antibiotic-resistant causing hospital-acquired infections.	Kristich *et al.*, 2011
	Arthrobacter creatinolyticus SM 15881T DSM	Human urine.	Nosocomial pathogen.	Hou *et al*.,1998
	Acinetobacter lwoffii 54 PIM	Normal flora of human (skin, upper respiratory tract and gastrointestinal tract, conjunctiva).	Potential human pathogen.	Patil *et al*., 2001
	Corynebacterium xerosis DSM 20743T DSM	Skin.	Pneumonia in a patient with acute leukemia.	Wallet *et al*., 1994
	Pseudomonas orientalis	Spring water.	Not reported.	Dabboussi *et al*., 1999

Areas	Organism	Isolated from	Implications	References
	CIP 105540T HAM			
	Lactobacillus sp DSM 20183 DSM	Sausage and the bovine rumen.	Used as probiotics in controlling colo-rectal cancer.	Gayathri and Devaraja, 2011
	Staphylococcus xylosus DSM 20266T DSM	Naturally-fermented sausages.	Starter culture for use in sausage production.	Di Maria *et al.,* 2002
Processing area 1	*Escherichia coli MB114641 CHB Escherichia coli ATCC 25922 THL*	Intestines of warm blooded organisms; urine and clinical specimens.	Urinary tract infections, widely used as a control for Gram-negative bacterium for various laboratory experiments.	Mandal and Brandl, 2011
	Arthrobacter citreus IMET 10680T HKJ	Chicken faeces.	Contaminant from dust or soil.	Sacks, 1954
	Lactobacillus curvatus DSM 20019T DSM	Milk and fermented meat products, vacuum-packaged sauerkraut, and silage.	Spoilage of refrigerated meat products.	Torriani *et al*., 1996
	Chryseobacterium joostei LMG 18212T HAM	Raw milk and dairy environment.	Cause a variety of defects in food products such as milk, meat, poultry and fish	De Beer *et al*., 2005; De Beer *et al*., 2006
	Citrobacter koseri 9553_1 CHB	Water, sewage, soils and food, as well as human faeces, urine, sputum, and other clinical specimens and animals.	Cause of sepsis and meningitis leading to central nervous system (CNS) abscesses in neonates and young infants.	Doran, 1999
	Aureobasidium pullulans 15131 CBS	Environments with fluctuating water activities such bathrooms, food and feeds areas.	Used for the packaging of food and drugs; producer of the biodegradable extracellular polysaccharide (EPS) pullulan (poly-α-1, 6-maltotriose).	Singh *et al*., 2008
	Arthrobacter sulfureus DSM	Oil brine.	Used in the process of microbial	Labana *et al.,* 2005

Areas	Organism	Isolated from	Implications	References
	20167T DSM		desulphurization significantly reducing the sulphur content of oil to help in meeting the regulatory standards for sulphur level in diesel oil.	
	Bacillus pumilus DSM 1794 DSM	Soil and aquatic environments.	Food spoilage and skin infections in immuno-compromised humans. Reduction of plant roots infection, and a pesticide. Produces an antibiotic pumilin.	Tena *et al.,* 2007
Processing area 2	*Arthrobacter sulfureus DSM 20167T DSM*	Oil brine.	Used in the process of microbial desulphurization significantly reducing the sulphur content of oil to help in meeting the regulatory standards for sulphur level in diesel oil.	Labana *et al.,* 2005
	Bacillus subtilis DSM 5611 DSM *Bacillus cereus 994000168 LBK* *Bacillus pumilus DSM 354 DSM*	Corn starch, soil and aquatic environments.	Food spoilage shortens shelf life and skin infections in immuno-compromised humans. Reduction of plant roots infection, and a pesticide. Produces an antibiotic pumilin.	Peltola *et al.*, 2001; Ozkocaman *et al.*, 2006; Tena *et al.,* 2007
	Corynebacterium variabile DSM 44702 DSM	Surface of smear-ripened cheeses.	Contributes to cheese ripening and quality, such as flavour formation.	Schroëder *et al.,* 2011
	Pseudomonas putida DSM 291T HAM	Soil, plants and water.	Causes septicaemia in immuno-compromised patients and nosocomial transmission.	Neulier *et al.,* 2011
	Kocuria carniphila DSM	Meat.	Not reported.	Tvrzová *et al*., 2005

Areas	Organism	Isolated from	Implications	References
	16004T DSM			
	Pantoea agglomerans CCM 4413 CCM	Plant surfaces, seeds, water, and humans (wounds, blood, urine, internal organs) and animals.	Cause galls and stalk and leaf necrosis on onions.	Gavini *et al.,* 1989
	Clostridium novyi 1082 ATCC 17861T BOG	Soil.	Cause of gas gangrene, malignant oedema in cattle and sheep.	Gorbach and Thadepalli, 1975
	Bacillus pseudomycoides DSM 12442T DSM	Soil.	Unknown.	Nakamura, 1998
	Acinetobacter schindleri DSM 16038T DSM	Human clinical specimen.	Rarely a human pathogen.	Nemec *et al.,* 2001; Nemec *et al.,* 2003
	Staphylococcus xylosus DSM 20267 DSM	Human skin and animals and naturally present in food.	Ability to form biofilms, starter culture for raw meat and milk fermentation.	Dordet-Frisoni *et al*., 2007a; Dordet-Frisoni *et al*., 2007b
	Lactobacillus sakei ssp. *carnosus DSM 15740 DSM Lactobacillus helveticus DSM 20075T DSM*	Meat and meat products. Human intestinal microflora and elemental cheese.	Important spoilage lactic acid bacteria of cooked meats (ropy slime). Used in the production of cheese preventing bitterness and to produce nutty flavours in the final cheese.	Slattery *et al*., 2010
	Macrococcus caseolyticus DSM 20597T DSM	Milk of cattle and goats including the abscesses of slaughtered lambs.	Non-pathogenic and pathogenic infections. Contribute to cheese maturation.	Kloos *et al.*, 1998
	Streptococcus phocae DSM 15635T DSM	Liver of seal.	Septicaemic condition (pneumonia).	Skaar *et al.*, 1994
	Corynebacterium falsenii DSM 44353T DSM	Blood specimen.	A potential human pathogen.	Bernard *et al.,* 2002

Areas	Organism	Isolated from	Implications	References
Display area	*Kytococcus sedentarius IMET 11362T HKJ*	Human skin flora.	Causative organism in various infections.	Stackebrandt *et al*., 1995
	Bacillus mojavensis DSM 9205T DSM *Bacillus pumilus DSM 354 DSM*	Desert soils and aquatic environments.	Endophytic bacterium patented for control of fungal diseases in maize and other plants. Food spoilage and skin infections in immuno-compromised humans. Reduction of plant roots infection, and a pesticide. Produces an antibiotic pumilin.	Peltola *et al*., 2001; Ozkocaman *et al.*, 2006; Tena *et al.*, 2007
	Pseudomonas aeruginosa ATCC 27853 CHB	Widely distributed in nature particularly in moist environments (hospital) and in antiseptic solutions.	Opportunistic pathogen causing infections in animals and humans and food spoilage.	Hare *et al*., 2012
	Paenibacillus lautus DSM 3035T DSM	Soil, water, rhizosphere, vegetable matter, forage and insect larvae, as well as clinical samples. Oral cavity of a dog.	Production of antibiotics.	Mead *et al.,* 2012
	Acinetobacter calcoaceticus 28 PIM *Acinetobacter sp DSM 30009 DSM*	Soil, blood and chicken.	Pneumonia due to ventilator.	Patil *et al*., 2001
	Campylobacter lari Cb 193_87 NVU	(Seabirds) isolated from gulls and puffins and from the environment.	Infrequent cause of intestinal and extra-intestinal infection in humans.	Brown *et al*., 2004
	Arthrobacter histidinolovorans DSM	Soil.	Characterized by the presence of threonine in the inter-peptide bridge of the peptidoglycan.	Koch *et al.*,1995

Areas	Organism	Isolated from	Implications	References
	20115T DSM			
	Micrococcus luteus N203 CPB	Soil, dust, water, air, normal flora of mammalian skin, colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman, and Peacock, 2007
	Citrobacter braakii 9314_2 CHB	Air, soil, sewage water.	Sepsis in a renal transplant recipient and opportunistic infections in immuno-compromised hosts.	Gupta *et al.*, 2003
	Brevundimonas diminuta DSM 7234T HAM	Freshwater, blood, patient with endocarditis.	Commonly used as a test organism for the efficiency of water filters due to the small size of the bacterium.	Lee *et al.*, 2002

Table 3.3: Isolated microorganisms from butchery B

Areas	Organism	Isolated from	Implications	References
Main entrance	*Acinetobacter lwoffii DSM 2403T DSM* *Acinetobacter lwoffii 2_Ring240 MHH* *Acinetobacter schindleri DSM 16038T DSM* *Acinetobacter baumannii ATCC 19606*	Normal flora of the skin, oropharynx and perineum of healthy individuals. Stagnant water sources, soil (dust). Human clinical specimens. Foods (vegetables, meat and fish), hospital environments.	Nosocomial pneumonia in immuno-compromised people Responsible for community-acquired meningitis and pneumonia via airborne transmission frequently misidentified opportunistic pathogen. Skin colonization.	Ku *et al.,* 2000; Nemec *et al*., 2001; Nemec *et al*., 2003
	Arthrobacter creatinolyticus DSM 15881T DSM	Human urine.	Urinary tract infections, bacteraemia, Whipple's disease.	Hou *et al*., 1998
	Streptococcus equinus DSM 20558T DSM	Pig intestines, chickens and horses faeces.	Opportunistic infections.	Schlegel *et al*., 2003
	Brachybacterium faecium IMET 11352T HKJ	Poultry deep litter.	Capable of degrading uric acid, and fermenting cellobiose, glucose, maltose, and mannose, but not cellulose, chitin, or gelatine.	Lapidus *et al.,* 2009
	Chryseobacterium joostei LMG 18212T HAM	Raw milk and dairy environment.	Causes a variety of defects in food products such as milk, meat, poultry and fish.	De Beer *et al*., 2005; De Beer *et al*., 2006
	Staphylococcus haemolyticus Mb18803_2 CHB	Throat, stool, blood, and tracheal aspirate.	Peritonitis, bloodstream infection.	Veach *et al*., 1990
	Bacillus megaterium DSM 32T	Soil, dried food, seawater,	The major aerobic producer of vitamin B	Vary, 1994

Areas	Organism	Isolated from	Implications	References
	DSM	sediments, fish, normal flora, and even in bee honey.	and is one of the organisms involved in fish spoilage.	
	Moraxella_sg_Moraxella osloensis 76 PIM	Human upper respiratory tract, skin and urogenital tract. Laundry environments.	Causes opportunistic infections and generates malodour in clothes.	Kubota *et al*., 2012
	Pseudomonas stutzeri B367 UFL	Soil and water including variety of body fluids and tissues.	Characterized as either the colonizing organism or a contaminant. Implicated in nosocomial and pseudo-outbreaks as well as vertebral osteomyelitis.	Reisler and Blumberg, 1999
	Arthrobacteroxydans DSM 20119T DSM Arthrobacter polychromogenes DSM 20136T DSM	Air, soil and arctic sea, beneath leaking radioactive waste tanks, and distilled water.	Used in the bioremediation of contaminated groundwater; Airborne infection.	Yotova *et al*., 2009; Schippers-Lammertse, 1963
	Kocuria rosea IMET 11363T HKJ	Rhizoplane of narrow-leaved cattail.	Peritonitis and bacteraemia episodes.	Kaya *et al.,* 2009
Processing area 1	*Campylobacter jejuni MB_6111_05 THL*	Poultry, unpasteurized milk and un-chlorinated water.	Diarrhoea (bloody and watery); fever, nausea headache and muscle pain.	Young *et al*., 2007
	Bacillus cereus 994000168 LBK	Soil, dust and cereal crops.	Food spoilage leading to food poisoning.	Guinebretière and Broussolle, 2002
	Corynebacterium callunae DSM 20147T DSM	Soil.	Glutamic acid-producing strain.	Fudou *et al*., 2002
	Cellulomonasgelida IMET 11078 HKJ	Soil.	Exhibit a constitutive chemotactic response toward cellobiose.	Hsing and Canale-Parola, 1992
	Acinetobactersp LMG 1300	Soil.	Resistant to many classes of antibiotics.	Rahal, 2006

Areas	Organism	Isolated from	Implications	References
	HAM			
	Micrococcus luteus BK_01140_09 ERL *Micrococcus luteus IMET 11249 HKJ*	Soil, dust, water, air, normal flora of mammalian skin, colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007
	Arthrobacter psychrolactophilus DSM 15612T DSM	Soil.	Produce enzymes having optimum activity at moderate to low temperature.	Loveland-Curtze *et al*., 1999
	Staphylococcus simulans DSM 20723 DSM	Animal (cattle, sheep) and their products also human skin.	Common animal pathogen and causes vertebral osteomyelitis and endocarditis, urinary tract infection in humans.	Vallianou *et al.*, 2008
	Aureobasidium pullulans 15131 CBS	Environments with fluctuating water activities such bathrooms, food and feeds.	Used for the packaging of food and drugs, producer of the biodegradable extracellular polysaccharide (EPS) pullulan (poly-α-1, 6-maltotriose).	Singh *et al*., 2008
Processing area 2	*Bacillus nealsonii DSM 15077T DSM* *Bacillus amyloliquefaciens CIP 103265T CIP*	Dust particles collected at a supercraft assembly facility and natural fall-out particle of the clean room air and soil.	Spores resistant to UV and gamma radiation, desiccation and H_2O_2. Responsible for production of a-amylase and protease.	Venkateswaran *et al*., 2003; Wang *et al*., 2008
	Serratia liquefaciens CCM 2716 CCM	Plant's rhizosphere.	Nosocomial infections due to poor hygiene.	Harnett *et al*., 2001
	Arthrobacter pascens DSM	Soil and medieval wall painting.	Not reported.	Altenburger *et al*.,

Areas	Organism	Isolated from	Implications	References
	20545T DSM			2002
	Micrococcus luteus IMET 11249 HKJ	Soil, dust, water, air, normal flora of mammalian skin, colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis and meningitis.	Bannerman and Peacock, 2007
	Enterobacter cloacae MB11506_1 CHB	Human skin and tissues as well as fruits, vegetables and water treatment tank devices, food animals such as ground beef cattle farm, processing facilities and clinical settings.	Important nosocomial pathogens responsible for various infections, including bacteraemia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections(UTIs), endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, CNS, and ophthalmic infections.	Musil *et al*., 2010
	Acinetobacter schindleri DSM 16038T DSM	Human clinical specimens.	Not reported.	Nemec *et al*., 2001; Nemec *et al*., 2003
	Kocuria palustris DSM 11925T DSM	Narrow-leaved cattail (*Typha angustifolia*).	Resistant to metallic and copper surfaces.	Savini *et al*., 2010
	Campylobacter coli 11167_03 NVU	Pigs and the production and processing environment at the farm and slaughtering facility.	Gastrointestinal campylobacteriosis. Resistant to a great number of antimicrobials.	Gürtler *et al.,* 2005
	Kocuria marina DSM 16420T DSM	Marine sediment.	Etiologic agents in various infections including peritonitis, a brain abscess in a diabetic patient, central venous	Lee *et al*., 2009

Areas	Organism	Isolated from	Implications	References
Display area			catheter-related.	
	Lactobacillus plantarum DSM 20246 DSM Lactobacillus acidipiscis DSM 15353 DSM	Fermented food products (fish, Halloumi cheese).	Probiotic properties and ferment sugar sintolactic acid (acid-loving milk-bacterium).	Salminen *et al*., 2006
	Klebsiella pneumonia ssp pneumoniae 9295_1 CHB	Normal flora of the mouth, skin, and intestines.	Opportunistic pathogens in nosocomial infections in patients.	Sabota *et al.,* 1998
	Microbacterium barkeri DSM 20145T DSM	Raw domestic sewage, the surface of the smear ripened cheeses.	Production of biodegradable plastics.	Shivakumar, 2012
	Viridibacillus arvi DSM 16317T DSM	Soil	Spore-forming spoilage organism.	Heyrman *et al.*, 2005; Ivy *et al.*, 2012
	Bacillus safensis CIP 109412 CIP	Clean room air particle, spacecraft assembly facility.	Highly resistant to gamma and UV radiation.	Satomi *et al*., 2006
	Acinetobacter johnsonii DSM 6963T DSM	Soil, water, sewage, milk products, meat, poultry and human skin.	Vascular catheter-related bloodstream infection.	Seifert *et al.,* 1993
	Neisseria meningitidis 24086406 MLD	Nasopharynx.	Transmitted to the host via airborne route causing upper respiratory tract infections, headache and death.	Madigan *et al.*, 2009
	Agrobacterium tumefaciens DSM 30147T HAM	Gall tissue, soil or water.	Cause crown gall and induce tumour.	Klapwijk *et al*., 1976
	Staphylococcus capitis ssp capitis DSM 6180 DSM	Human and animal skin. Surface ripened cheese and	Endocarditis, septicaemia to neonatal and nosocomial infection (rarely).	Place *et al*., 2002

Areas	Organism	Isolated from	Implications	References
	Staphylococcus succinus ssp casei DSM 15096T DSM 2008	dominican amber.		

Table 3.4: Isolated microorganisms from butchery C

Areas	Organism	Isolated from	Implications	References
Main entrance	*Kocuria marina DSM 16420T DSM*	Marine sediment.	Etiologic agents in various infections including peritonitis, a brain abscess in a diabetic patient, central venous cather-related.	Lee *et al.,* 2009
	Bacillus cereus 994000168 LBK	Soil, dust and cereal crops.	Food spoilage leading to food poisoning.	Guinebretière and Broussolle, 2002
	Brevundimonas vesicularis DSM 7226T HAM	Water and rarely isolated from clinical specimen	Can cause disease in a person without predisposing disease.	Bhatawadekar and Sharma, 2011
	Acinetobacter lwoffii 2_Ring240 MHH	Normal flora of the skin, oropharynx and perineum of healthy in individuals Stagnant water sources, soil (dust).	Nosocomial pneumonia in immuno-compromised people. Responsible for community-acquired meningitis and pneumonia via airborne transmission.	Ku *et al.,* 2000
	Arthrobacter oxydans IMET 10684T HKJ *Arthrobacter pascens DSM 20545T DSM*	Soil.	Degrade the phenylcarbamate herbicides phenmedipham and desmediphamco metabolically by hydrolyzing their central carbamate linkages. Play a part in adaptation to an environmental stress in a heterologous organism.	Pohlenz *et al*., 1992; Rozwadowski *et al*., 1991
	Staphylococcus equorum	Fermented sausages and skin	Contribute to the development of meat	Švec *et al*., 2004

Areas	Organism	Isolated from	Implications	References
	ssp. *equorum DSM 20674T DSM*	of healthy horses and nares of poultry and goats.	flavour.	
	Bacillus circulans DSM 11T DSM	Soil.	Wound infection in a patient with malignant ovarian carcinoma, meningitis, cerebrospinal fluid shunt infection, prosthetic heart valve, endocarditis, and endophthalmitis.	Alebouyeh *et al*., 2011
Processing area 1	*Microbacterium saperdae IMET 11076T HKJ*	Rhizosphere of zinc hyper-accumulatin plant elm borer (*Saperda carcharias*).	Can solubilize zinc.	Whiting *et al*., 2001
	Staphylococcus vitulinus DSM 9931 DSM	Isolated from human and animal specimens.	Novobiocin-resistant.	Švec *et al*., 2004
	Bacillus safensis CIP 109412 CIP *Bacillus cereus 4080 LBK*	Clean room air particle, spacecraft assembly facility. Soil, dust and cereal crops.	Highly resistant to gamma and UV radiation. Food spoilage leading to food poisoning.	Satomi *et al*., 2006; Guinebretière and Broussolle, 2002
	Arthrobacter globiformis DSM 20124T DSM *Arthrobacter psychrolactophilus DSM 15612T DSM*	Soil from whey-enriched farm field.	Bacteriophage in soil produces novelβ-galactosidases that are able to hydrolyse lactose at low temperature.	Loveland-Curtze *et al.*, 1999
	Kocuria palustris DSM 11925T DSM *Kocuria rhizophila DSM 11926T DSM*	Narrow-leaved cattail (*Typha angustifolia*). Soil (dust), mammalian skin, fermented foods, clinical specimens, fresh	Resistant to metallic copper surfaces.	Savini *et al.*, 2010

Areas	Organism	Isolated from	Implications	References
		water source and marine sediments.		
Processing area2	*Bacillus safensis CIP 109412 CIP* *Bacillus cereus DSM 31T DSM*	Clean room air particle, spacecraft assembly facility. Soil, fresh water, dairy products, food of animal and plant origin.	Highly resistant to gamma and UV radiation. Food spoilage leading to food poisoning.	Satomi *et al*., 2006
	Micrococcus luteus 59 PIM	Soil, dust, water, air, normal flora of mammalian skin, colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007
	Stenotrophomonas maltophilia (Pseudomonas ibiscicola) LMG 980T HAM	Water, soil, animals and plants.	Risk factors associated with *Stenotrophomonas* infection include HIV infection, malignancy, cystic fibrosis, neutropenia, mechanical ventilation.	Looney *et al*., 2009
	Massilia sp 992100145_2 LBK	Blood of an immuno-compromised patient with meningoencephalitis.	Not reported.	La Scola *et al.*, 1998
	Kocuria palustris DSM 11925T DSM	Narrow-leaved cattail (*Typha angustifolia*).	Resistant to metallic copper surfaces.	Savini *et al.*, 2010
	Staphylococcus lentus DSM 6672 DSM	Sheep, goat udder, raw milk urine of human and intestinal	Can cause goat mastitis and opportunistic pathogen in immuno-	Hauschild *et al.*, 2005

Areas	Organism	Isolated from	Implications	References
		tract of house fly larvae.	compromised patients.	
	Arthrobacter oxydans IMET 10684T HKJ	Human clinical specimen.	Opportunistic pathogens.	Whitman *et al.,* 2012
	Solibacillus silvestris DSM 12223T DSM	Forest soil.	Undetermined.	Rheims *et al.*, 1999
	Staphylococcus hominis Mb18788_1 CHB *Staphylococcus lugdunensis DSM 4804T DSM*	Human skin and clinical specimens.	Nosocomial, bloodstream infection and infective endocarditis and catheter-related infections.	Choi *et al.*, 2010
	Kocuria palustris DSM 11925T DSM	Rhizosphere of narrow-leaved cattail (*Typha angustiflora).*	Resistant to metallic copper surfaces.	Kovács *et al.*, 1999
	Bacillus subtilis ssp*. subtilis DSM 5660 DSM* *Bacillus cereus 994000168 LBK*	Soil (dust), air plant, water, temporary inhabitant of human skin and gastro-intestinal tract, faecal matter, fermented food products.	Supports plant growth, restores healthy bacterial communities in the body enhancing the immune system. Food spoilage leading to food poisoning.	Guinebretière and Broussolle, 2002
	Pantoea sp 110 PIM	Soil, water, seeds, animal and human wounds, blood and urine.	Opportunistic human pathogen.	De Champs *et al*., 2000

Table 3.5: Isolated microorganisms from butchery D

Areas	organism	Isolated from	Implications	References
Main entrance	*Bacillus pumilus DSM 354 DSM*	Soil and aquatic environments.	Food spoilage organism causes skin infections in immuno-compromised humans. Reduces infection of plant roots, and act as a pesticide. Produces an antibiotic called pumilin.	Tena *et al.*, 2007
	Micrococcus luteus IMET 11249 HKJ x2	Soil, dust, water, air, normal flora of mammalian skin, colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman, and Peacock, 2007
	Arthrobacter sulfonivorans DSM 14002T DSM	Soil from root balls of *Allium aflatunense.*		Borodina *et al*., 2002
	Kocuria rhizophila DSM 348 DSM	Rhizosphere of narrow leaf cattail, chicken meat treated with oxali cacid.	Additional member of suborder of *Micrococcineae* which are able to cause infections in humans.	Kovács *et al*., 1999
	Acinetobacter parvus DSM 16617T HAM	Ear of a dog.	Nosocomial infections.	Nemec *et al*., 2001; Nemec *et al*., 2003
Processing area 1	*Bacillus megaterium DSM 32T DSM*	Soil to seawater, sediment, rice paddies, honey, fish, and dried food.	Production of exoenzymes and cloning host for the production of intact proteins.	Bary, 1884
	Acinetobactersp LMG 1138 HAM	Soil.	Degrade the sodium acrylate oligomer and utilize it as the sole source of	Hayashi *et al*., 1993

Areas	organism	Isolated from	Implications	References
			carbon.	
	Micrococcus luteus IMET 11249 HKJ	Soil, dust, water, air, normal flora of mammalian skin, colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007
Processing area 2	*Escherichia coli DH5alpha BRL*	Meat and meat products.	Antimicrobial resistance and the impact on human health.	Sunde and Norström, 2006
	*Aeromonas hydrophila*ssp. *anaerogenes DSM 30188T HAM*	Aquatic environment.	Not reported.	Dudley and Churchill, 1995
	Wautersiella falsenii 02_08_TR IBS	Surgical wound, urine of an infant with a complicated urinary tract infection.	Potential pathogens isolated from metalworking; fluids or aerosols.	Van der Velden *et al.,* 2012
	Streptococcus parauberis DSM 6631T DSM	Cattle, fish and water.	Etiologic agent of bovine mastitis; Streptococcus in fish.	Currás *et al.,* 2002
	Arthrobacter mysorens DSM 12798T DSM	Soil, sewage and skin.	Skin erythemas.	Imirzalioglu *et al.*, 2010
	Moraxella_sg_Moraxellaoslo ensis 76 PIM	Human upper respiratory tract; skin and urogenital tract; laundry environments.	Causes opportunistic infections, blood or catheter infections, and generates a malodour in clothes.	Kubota *et al*., 2012
	Kocuria rhizophila DSM 11926T DSMx3	Rhizosphere of narrowleaf cattail, chicken meat treated with oxalic acid.	Additional member of suborder of *Micrococcineae* which are able to cause infections in humans.	Kovács *et al*., 1999

Areas	organism	Isolated from	Implications	References
Display area	*Staphylococcus lutrae DSM 10244T DSM*	Otter'stissue/muscles (mammary gland and supra mammary lymph node).	Human infections.	Kwok and Chow, 2003
	Arthrobacter histidinolovorans DSM 20115T DSM	Soil.	Metabolic intermediate.	Adams, 1995
	Staphylococcus xylosus DSM 6179 DSM	Human and animal, naturally present in raw meat and milk skin.	Starter culture for fermented meat products. Ensures colour development and contributes to aroma.	Dordet-Frisoni *et* Dordet-Frisoni *et al.*, 2007a *al.*, 2007b
	Acinetobacter sp DSM 30012_DSM Acinetobacter lwoffii DSM 2403T DSM	Normal flora of the skin, oropharynx and perineum of healthy individuals; stagnant water sources, soil (dust).	Nosocomial pneumonia in immuno-compromised people; responsible for community-acquired meningitis and pneumonia via airborne transmission.	Ku *et al.,* 2000

Table 3.6: Isolated microorganisms from butchery E

Areas	Organism	Isolated from	Implications	References
Main entrance	*Bacillus cereus 994000168 LBK* *Bacillus safensis CIP 109412 CIP*	Spacecraft assembly facility.	Highly resistant to gamma and UV radiation.	Satomi *et al*., 2006
	Acinetobacter tandoii DSM 14970T HAM	Isolated from activated sludge.	Not known.	Carr *et al.*, 2003
	Neisseria meningitidis 24086406 MLD *Neisseria meningitis serogroup-YBRL*	Nasopharynx.	Transmitted to the host via airborne route causing upper respiratory tract infections, headache and death.	Takahashi *et al.*, 2012; Madigan *et al*., 2009
	Micrococcus luteus IMET 11249 HKJ x4	Soil, dust, water, air, normal flora of mammalian skin; colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007
	Kocuria palustris DSM 11925T DSM	Rhizosphere of narrow-leaved cattail (*Typha angustiflora).*	Resistant to metallic copper surfaces.	Kovács *et al*., 1999
	Staphylococcus aureus ssp aureus DSM 20491 DSM	Faecal matter, foods, soil, normal flora of human intestines.	Food poisoning and variety of diseases.	Švec *et al*., 2004
	Micrococcus luteus BK_01140_09 ERL *Micrococcus luteus IMET 11249 HKJ*	Soil, dust, water, air, normal flora of mammalian skin; colonizes human mouth, oropharynx and upper	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007

Areas	Organism	Isolated from	Implications	References
Processing area 1		respiratory tract.		
	Kocuria rosea IMET 11363T HKJ	Soil and water.	Opportunistic pathogen in the immuno-compromised patient.	Altuntas *et al*., 2004
	Staphylococcus vitulinus DSM 9930 DSM	Human hip infection.	Novobiocin-resistant and oxidase-positive species.	Švec *et al*., 2004
	Corynebacterium callunae DSM 20147T DSM	Soil.	Produces large amounts of L-glutamic acid.	Fudou *et al*., 2002
	Acinetobacter baumannii ATCC 19606	Soil, foods (vegetables, meat and fish). Hospital environments and water sources.	Nosocomial pneumonia infections, skin colonization.	Horii *et al*., 2011
	Micrococcus luteus IMET 11249 HKJ	Soil, dust, water, air, normal flora of mammalian skin; colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007
	Arthrobacter chlorophenolicus DSM 12829T DSM	Soil.	Capable of degrading high concentrations of 4-chlorophenol and useful in bioremediation.	Westerberg *et al*., 2000
	Kocuria palustris DSM 11925T DSM	Rhizosphere of narrow-leaved cattail (*Typha angustiflora).*	Not reported.	Kovács *et al.,* 1999
	Escherichia coli DH5 alpha BRL	Intestines of warm blooded organisms; urine and clinical specimens.	Urinary tract infections.	Ferreira *et al*., 1990; Whyte *et al*., 2001

Areas	Organism	Isolated from	Implications	References
Processing area	*Moraxella_sg_Moraxellaosloensis 76 PIM*	Human upper respiratory tract, skin and urogenital tract; laundry environments.	Cause opportunistic infections and generate a malodour in clothes.	Kubota *et al*., 2012
	Micrococcus luteus 59 PIM	Soil, dust, water, air, normal flora of mammalian skin; colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007
	Burkholderia thailandensis DSM 13276T HAM	Rice field sample in Thailand and environmental samples such as soil.	Distribute potential virulence genes with *B. mallei* and *B. pseudomallei.*	Brett *et al.,* 1998
	Arthrobacter polychromogenes DSM 20136T DSM *Arthrobacter tecti DSM 16407T DSM*	Air and biofilm overgrowing on the ceiling.	Airborne infections and biodeterioration of mural paintings.	Schippers-Lammertse *et al*., 1963 Heyrman *et al*., 2005
	Staphylococcus vitulinus DSM 9931 DSM	Human clinical specimen and animals, calf, horse, fish and meat products.	Pathogenicity unknown but a potential for nosocomial infections and food contamination.	Švec *et al.,* 2004
	Staphylococcus equorum ssp equorum DSM 20674T DSM	Human and animal skin of horse, fermented sausages, curing brine and raw ham.	Contribute to the development of meat.	Švec *et al*., 2004

Areas	Organism	Isolated from	Implications	References
Display area	*Klebsiella pneumoniae 37924 PFM*	Human intestines, patients whose care requires devices like ventilators (breathing machines), human stool.	Cause respiratory disease: pneumonia, urinary tract infection.	Bolister *et al.*, 1992
	Wautersiella falsenii 02_08_TR IBS	Surgical wound and blood; urine.	Urinary tract infections.	Van der Velden *et al.,* 2012
	Micrococcus luteus IMET 11249 HKJ	Soil, dust, water, air, normal flora of mammalian skin; colonizes human mouth, oropharynx and upper respiratory tract.	Intracranial abscesses, pneumonia, septic arthritis, endocarditis, and meningitis.	Bannerman and Peacock, 2007
	Kocuria rosea DSM 20447T DSM	Rhizoplane of narrow-leaved cattail.	Pathogen causing catheter-related bacteremia and acute cholecystitis.	Altuntas *et al*., 2004
	Pseudomonas fulva 013_W30 NFI	Rice and cerebrospinal fluid.	Meningitis in human.	Almuzara *et al*., 2010
	Acinetobacter ursingii DSM 16037T HAM	Human clinical specimens, blood of female with endocarditis.	Bacteremia, blood stream infections.	Horii *et al.,* 2011
	Citrobacter freundii 22054_1 CHB	Distributed in soil, water and the intestines of humans and animals.	Pathogen in food capable of colonizing human skin and intestine.	Badger *et al*., 1999
	Lactobacillus vitulinus DSM 20405T DSM *Lactobacillus saerimneri DSM*	Calf, rumen; pig, faeces.	Genotypically heterogenous, minor component in the gastrointestinal microbiota of pigs.	Sharpe and Dellaglio, 1977; Pedersen and Roos, 2004

Areas	Organism	Isolated from	Implications	References
	16027 DSM			
	Staphylococcus xylosus DSM 20266T DSM	Human and animal, naturally present in raw meat and milk skin.	Starter culture for fermented meat products. Ensures colour development, contributes to aroma.	Dordet-Frisoni *et al.*, 2007a; Dordet-Frisoni *et al.*, 2007b

spore formers with the ability to disperse rapidly through the air and are ubiquitous in the environment, occurring as saprophytes in water and soil (Nicholson *et al*., 2002; Vilain *et al*., 2006). *Bacillus cereus* and *B. subtilis* can, to a lesser extent, be pathogenic in humans and mammals. A study conducted by Merrill *et al*. (2006) suggests that the presence of *Bacillus* spp. in the air contaminating food products may be attributed to the aerosolization of soil particles, wind, as well as the amount of moisture in the air (i.e. relative humidity).

In the meat industry, *Bacillus* spp. have been reported not to be a spoilage problem; however, spores of these organisms may be introduced to meat through other ways such as handling, addition of spices, poor hygiene status, amongst others (Doyle, 2007; Fernandes, 2009). *Bacillus cereus,* which is considered the most problematic of the *Bacillus* genus, was the species mostly isolated of the genus at butcheries; and with its ability to produce enterotoxin, became a huge concern (Phelps and McKillip, 2002). The incidence of *Bacillus cereus* in cooked and processed meat is higher than in raw meat samples (Nortjé *et al.,* 1999; Mosupye and Von Holy, 2000). Spores of *B. cereus* and *B. subtilis* survive cooking and can subsequently germinate and grow when conditions are favourable. *Bacillus subtilis* was the second most isolated *Bacillus* spp. (Tables 3.2 and 3.4). This species are considered non-pathogenic and can result in food contamination and spoilage; however, they are seldom reported to cause any food poisoning (Jagannath *et al.,* 2005).

Apart from *Bacillus* spp, *Staphylococcus* is ubiquitously distributed in nature and known to be the normal flora on the skin, hair and mucous membrane of both humans and animals. However, *Staphylococcus* spp. can cause infections when they are introduced to normally sterile parts of the body and are considered agents of opportunistic diseases in animals and human (Fidalgo *et al.,* 1990). In food, *Staphylococcus* has previously been isolated from a variety of foodstuffs including meat products (Shale*,* 2004; Goja *et al.,* 2013).

A variety of staphylococci strains isolated from this study were commonly found in the environment as an integral part of the natural flora (Irlinger, 2008). The isolated *Staphylococcus* strains included *Staphylococcus equorum* ssp. *equorum* DSM 20674T DSM, *Staphylococcus haemolyticus* Mb 188032 CHB*, Staphylococcus hominis* Mb 187881 CHB*, Staphylococcus capitis* ssp. *capitis* DSM 6180 DSM*, Staphylococcus vitulinus* DSM 9930 DSM*, Staphylococcus vitulinus* DSM 9931 DSM, *Staphylococcus lugdunensis* DSM 4804T DSM*, Staphylococcus lutrae* DSM 10547 DSM*, Staphylococcus lentus* DSM 6672 DSM*, Staphylococcus succinus* ssp. *casei* DSM 15096T DSM*, Staphylococcus simulans* DSM 20723 DSM*, Staphylococcus xylosus* DSM 20266T DSM, and *Staphylococcus xylosus* DSM 6179 DSM. The presence of all isolates in food has never been reported to result in the spoilage; rather, their presence has been reported for their ability to cause infections (Tables 3.2–3.6). Staphylococcal strains isolated in the current study are important pathogens related to food poisoning. *Staphylococcus* species identified by Holt *et al*. (1994) and Shale (2004); indicated a high prevalence of these species in the receiving area of meat processing plants as

result of dispersion from food handlers' skin and clothes into the air. This is in agreement with the findings of the current study as most of the isolated strains were from human or animal origin (Tables 3.2–3.6).

The next group is the genus *Campylobacter* which is spiral or S-shaped, non-spore-forming, micro-aerophilic Gram-positive bacteria that are recognized to cause campylobacteriosis, which is one of the leading causes of foodborne illness in industrialized countries (Allos, 2001). *Campylobacter* are sensitive to a variety of external physical conditions such as low water activity, ultraviolet light, and heat and salt. Species from this genus do not multiply outside warm-blooded hosts because of the absence of micro aerobic conditions and non-permissive temperatures [European Food Safety Authority (EFSA), 2011]. However, *Campylobacter* spp. can survive in the environment for extended periods particularly when relative humidity is high, resulting in a potential to become aerosolized (Nicholson *et al.,* 2005).

The main agent causing human illness is *Campylobacter jejuni,* but other species such as *C. coli, C. lari and C. upsaliensis* can also cause disease in humans (Table 3.2 and 3.3). These species occur naturally in the gastrointestinal tract of domestic and live animals; however, they are considered to be the leading cause of bacterial foodborne diarrhoeal disease globally (Silva *et al.,* 2011). The presence of these microorganisms at butcheries indicates inadequate hygiene practices such as utilizing the same cutting equipment without proper sanitation and lack of proper hand-washing practices. Incidence of *Campylobacter* in the meat industry have been found to occur as a result of

under-cooking and cross-contamination from raw to cooked meat (Sampers *et al.,* 2012).

Moreover, *Lactobacillus* genus on the other hand occurs widely in nature and constitutes a major part of lactic acid bacteria, with a large range of physiochemical, biochemical and phenotypic properties. Members of the *Lactobacillus* genus, among other Gram-positive bacteria, are safely used as starter cultures in various foods and are consumed daily by normal as well as immune-compromised humans. However, *L. plantarum* and *L. acidophilus, L. rhamnosus, L. fermentum, L. casei, L. jensenii, L. salivarius, L. gasseri,* and *L. Salivarius* have also been isolated from human clinical specimens and associated with endocarditis and bacteraemia (Salminen *et al*., 2006).

Furthermore, another group is *Streptococcus* species that are a group of bacteria that are frequently isolated from the upper respiratory tract and skin of humans. Streptococci can be spread through direct contact with wounds on skin or mucous from the nose or throat of an infected person causing necrotizing fasciitis, streptococcal toxic shock syndrome. *S. paraubesis,* isolated in butchery D (Table 3.2), is an important mastitis-causing pathogen in dairy cattle; it also causes *Streptococcus* disease in fish and in vacuum-packed food products (Fernández-No *et al*., 2012). Thus, the species poses a risk to public health through the dairy and aquaculture industries (Nho *et al*., 2011). The understanding of *S. paraubesis* is therefore of vital importance to avoid global economic loss, and also for the maintenance of animal health because of the dependence of

humans on food of animal origin. Significantly, the isolation of *S. paraubesis* in butcheries indicates poor personal hygiene on the part of meat handlers.

In addition to the other groups above, members of the *Kocuria* genus are widely isolated from natural sources including the rhizosphere of narrow-leaved cattail, soil, fresh water, marine sediments, human and mammalian skin, and clinical specimens of fermented foods (Kovacs *et al*., 1999). The majority of the *Kocuria* strains are non-pathogenic although some strains may be opportunistic pathogens. They are created from the genus micrococcus, and *K. rhizhopila* is important in industrial application.

Similarly to *Staphylococcus* group, members of the genus *Micrococcus* are also present as normal flora of human and mammalian skin and mucosa. Although infections related to *Micrococcus spp.* are uncommon, they are normally recognized in immune-compromised patients with other contributory diseases. Furthermore, *Micrococcus* spp*.* are associated with central venous catheter infection in patients with pulmonary hypertension receiving continuous epoprostenol infusion (Oudiz*,* 2004). In the current study, *M. luteus* was isolated in all the butcheries (shown in Tables 3.2–3.6) and has been described as the causative agent in meningitis (Fosse *et al*., 1985), intracranial abscess, arthritis, pneumonia and catheter-related sepsis in patients undergoing haemodialysis (Bannerman and Peacock, 2007). *Micrococcus luteus* are commonly found in various environments and therefore are typical airborne microorganisms (Miller and Macher, 2000; Agranovski *et al.,* 2003).

Apart from the above groups, pathogenic Gram-negative bacteria from the environment can affect the safety and wholesomeness of meat products through airborne contamination. Moreover, Gram-negative bacteria have a long history of causing infections in both humans and animals. In the current study, Gram-negative bacteria that are normally associated with human infections were isolated in butchery environments. An example is *Neisseria meningitidis* which is a strict human pathogenic member of the *Neisseriae* family. These coccoid-shaped, Gram-negative bacteria were previously isolated from the cerebrospinal fluid of patients with meningitis (Weichselbaum, 1887). In endemic areas, *Neisseria meningitidis* is known to colonize the nasopharyngeal mucosa and the throat without affecting the host in approximately 10% of the healthy population (Cartwright *et al.*, 1987; Stephens, 1999). Antibodies built up by the body prevent the spread of this organism to other parts of the body (Kremastinou *et al.,* 1999). Transmission of this organism occurs from person-to-person via respiratory droplets generated primarily during coughing, sneezing or talking, and is normally increased amongst closed populations such as military recruits, university students' residences and/or halls, as well as in household contacts in cases of meningococcal infection (Olcén *et al.,* 1981; Cartwright, 1995).

The other group is *Pseudomonas* spp. That was also one of the dominant Gram-negative bacteria isolated in Tables 3.2, 3.3 and 3.6. These bacteria are widely distributed in water, soil and air, with some strains reported as important animal pathogens (Palleroni, 1992). The occurrence of *Pseudomonas* spp. in fresh food including meat is well documented (Gill *et al*., 1996; Gill, 1996). Although the species is

not an important causative agent of spoilage in processed meat, when vacuum-packed meats are opened after insufficient heating, pasteurization or curing, these bacteria can potentially spoil refrigerated processed meats (Ercolini, 2006). Thus, *Pseudomonas* are psychrophilic spp. because of their ability to grow and spoil fresh meat stored at chilled temperatures (Aberle *et al.,* 2001). According to Marthi *et al*. (1990), aerosolized *Pseudomonas* spp. proliferation can be achieved at high relative humidity and low temperature.

Escherichia coli on the other hand belong to the family *Enterobactericea* and the isolation of this bacterium in this study is a clear indication of faecal contamination and indicator of enteric pathogens present in the butcheries (South Africa: Department of Health, 2000). Although these bacteria are normal flora in the intestinal tract of humans and various animals, some of the strains are virulent with a potential of causing fatalities. In the current study, *E. coli* was predominately distributed in the processing areas as shown in Tables 3.2; 3.4; 3.5 and 3.6. A similar study conducted by Whyte *et al*. (2001) revealed higher counts of *Escherichia coli* from the de-feathering and evisceration areas, which suggests a need for separation of the poultry areas into clean and dirty since air was identified as a carrier of pathogenic bacteria. In butcheries A and D, the suspension of *E. coli* in the air could have been greatly influenced by the close proximity of toilets near the processing areas, by workers flushing the toilets (Mandal and Brandl, 2011). Moreover, the isolation of these organisms in the present study could be due to pure negligence in terms of poor hand-washing and poor sanitation of the plant butcheries and equipment.

Lastly, members of the genus *Acinetobacter* have emerged as significant nosocomial pathogens (Kurcik-Trajkovska, 2009; Doughari *et al.,* 2011). In the present study they were observed as a frequently occurring genera present in all the sampled butcheries (Tables 3.2-3.6). *Acinetobacter* species are non-motile, Gram-negative coccobacillus, implicated as spoilage organisms in various foodstuffs (meat, poultry, fish and milk products) and are widespread environmental contaminants occurring from sources such as soil, water and sewage, amongst others (Bernards *et al*., 2004). From the isolated *Acinetobacter* spp., *A*. *baumannii* caused concern in the current study as this species has been reported to be resistant to antibiotics, posing a formidable threat to, and causing a high mortality rate among hospital patients (Lee *et al.,* 2011; Savov *et al.,* 2002). In a study conducted by Wilks *et al.* (2006), circumstances that led to the outbreak of multi-drug resistant *Acinetobacter baumannii* colonization and infection in an intensive care unit included their presence on medical equipment, door handles, mops, cell-phones and keyboards. Therefore, the occurrence of *Acinetobacter* spp. in sampled butcheries is a potential health hazard to exposed workers as well as to the safety and quality of food (Tables 3.2–3.6).

3.5 Conclusions

Airborne microorganisms are considered to be a hazard in indoor environments when present in high concentrations as they could result in the spoilage of food as well as a wide range of effects in humans. According to several studies, different levels of airborne contaminants in food environments (including the meat industry) have been observed (Kang and Frank, 1990; Ellerbroek, 1997, Lutgring *et al.,* 1997; Whyte *et al*.,

2001; Cundith *et al.,* 2002; Shale, 2004; Sutton, 2004; Lues *et al.,* 2007). Although a number of studies have been done on airborne contaminants in the meat processing industry, little is known about airborne contamination in butchery environments.

In the current study, the levels of airborne contaminants in one of the butcheries with lowest counts reflected a minimum of 2×10^0 and maximum of 3.77×10^2 cfu.m^{-3}. On the other hand, the butchery with high counts recorded minimum of 2.7×10^2 and a maximum of 5.41×10^3 cfu.m^{-3}. Although no agreed standard of microbial counts exists, the counts found in the present study were considerably higher than the counts found in the study conducted by Shale (2004) in the red meat abattoirs where counts ranged between 1.3×10^2 and 3.1×10^2 cfu.m^{-3} (especially when comparing with the butchery with highest counts).

Exposure to airborne contaminants in indoor environments is virtually inevitable as these contaminants are ubiquitous in nature. In this study, a variety of Gram-positive and Gram-negative bacteria were isolated in the various sections of the butcheries. The frequently isolated Gram-positive airborne bacteria included *Bacillus*, *Staphylococcus* and *Micrococcus* species which are known to cause spoilage in food. Moreover, these microbes are known as indicators of faecal contamination which suggest poor handling and hygiene status. The presence of this species could be attributed to human and animal skin, soil, dust and water. In addition, Gram-negative airborne bacteria isolated were mostly from the *Enterobacteriaceae* and *Pseudomonadaceae* family. Airborne

contaminants identified from the different sections of the butcheries in this study reaffirm the fact that internal sources such as movement of employees, mechanical ventilation systems, dust and sanitation procedures play a substantial role in the transportation and distribution of these microbes, largely contributing to the possible contamination of meat and meat products, and therefore posing a microbial hazard to public health. This study revealed a need to measure the levels of suspended dust and bioaerosols and to identify the taxa distributions of pathogenic and non-pathogenic airborne contaminants.

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CHAPTER 4

Bacteriological analysis of environmental surfaces in butcheries

BACTERIOLOGICAL ANALYSIS OF ENVIRONMENTAL SURFACES IN BUTCHERIES

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4.1 Abstract

Despite the importance of food hygiene and safety within the food processing environment, a high prevalence of foodborne illnesses linked to products produced under poor sanitation conditions continue to be reported. In this study, environmental surface samples were collected from knives, bowl cutters, band saws, sausage fillers and floors in the selected butcheries in the Mangaung Metropolitan municipal area. Total Viable Counts (TVC) were determined for each sampled surface and identified with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Average surface counts ranged between 5.12×10^{0} cfu.cm^{-2} and 1.96×10^{2} cfu.cm^{-2}. The total bacterial counts on surfaces at the butcheries over the entire duration of the study ranged from 3.24×10^{3} cfu.cm^{-2} to 6.57×10^{3} cfu.cm^{-2}. The most prominent microbial isolates identified in the study were *Bacillus, Pseudomonas*, *Lactobacillus*, *Staphylococcus*, *Kocuria*, *Acinetobatcter*, *Micrococcus*, *Escherichia coli,* and *Neisseria*, amongst others. The findings of this study indicate that meat processing equipment might be a hazard to meat products as some of the isolated microbial species are known as indicator organisms and food spoilers from biofilm communities. In conclusion, emphasis should also be placed on the mandatory implementation of HACCP or related food management systems to ensure food safety as well as on the introduction and promotion of general food safety management systems.

Keywords: *Environmental surfaces, butcheries, food safety, contamination*

4.2. Introduction

Hygiene is considered to be one the critical aspects for the survival and maintenance of butchery establishments. However, because most local health departments utilize visual inspection to assess surfaces rather than microbiological inspection, the establishment of the level of hygiene and microbial contamination has proven to be insufficient (Griffith *et al.,* 2000; Attala and Kassem, 2011). In recent years, harmful bacteria and disease outbreaks related to food of animal origin have been reported as a result of cross-contamination (Evans *et al.,* 1998; Pennington, 2009; Ali *et al*., 2010). The healthy animal inner tissue is considered sterile though contamination may be introduced during further processing of meat into desired cuts (Van der Walt, 2005). Moreover, meat surfaces have been reported to harbour numerous bacterial species that are known to cause spoilage (Montville and Matthews, 2007). Contamination of meat may pose a risk of transmitting foodborne illnesses or zoonosis, leading to product recalls and loss of consumer confidence in the safety and quality of meat, resulting in public health concern (Bhandare *et al*., 2007; Bhandare *et al*., 2009).

Although government health officials throughout the world have the responsibility of improving the safety of food by inspecting the food premises, millions of people continue to be presented with various diseases as a result of consuming contaminated food (Tavakoli and Riazipour, 2008; World Health Organization, 2011). Microorganisms are small and cannot be detected easily by the naked eye. Consequently, meat contact

surfaces that are not adequately cleaned and microbiologically tested may lead to biofilm formation and survival of chemical residues on the meat processing equipment (Whitehead *et al*., 2010). Generally, the efficient way of preventing microbial contamination and minimizing microbial growth in the raw to final processed meat is through the application of management tools such as good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) amongst others (Attala and Kassem, 2011). However, the HACCP system as a tool for preventing, eliminating and reducing health hazards is not mandatory in South Africa (South Africa, Department of Health, 2003). This is in contrast to the United States of America where the system is mandatory and designed to ensure the general safety and quality of food including meat and meat products: liability lies with the meat processors (Goodrich *et al.,* 2010).

The aim of this study was to investigate the microbial load on meat processing surfaces in the Mangaung Metropolitan Municipality butchery environments in selected butcheries. The species isolated were identified and fingerprinted using MALDI-TOF MS, and attempts were made to establish main points of contamination and possible origins of the microbial contamination. Furthermore, this study was conducted to provide evidence to support and/or challenge the existing bodies of knowledge regarding the practices of food handlers and the level of hygiene in butcheries in relation to food quality and the possible health effects in humans.

4.3. Materials and methods

4.3.1 Sampling site

The study was conducted in five selected retail outlets (forming 15% of total registered butcheries at the time the study was conducted), three of which were in the central business district (CBD) and two in the township of Mangaung. Some of the butcheries were chosen as result of reports of non-compliance with meat safety regulations. The butcheries were visited five times over a period of three months on a fortnightly basis for all analysis.

4.3.2 Sample protocol

Samples were obtained at least in duplicate from eighteen environmental surfaces with the use of swabs (Merck, South Africa). Samples were taken from meat processing equipment such as knives, weighing scales, meat mincers, bowl cutters, band saw machine surfaces and blades, hooks, sealers, sinks, scoops, meat containers, trays, floors and tables, meat slicer blades and surfaces. Samples were collected in the early hours of the morning post cleaning and prior to commencement of meat processing. It must also be stated that some butcheries did not have all or similar equipment due to their various needs but at least more than 90% of the items were found in all butcheries. All of the items per butchery are reported in this study although they may not be available at some of the butcheries in order to capture possible contamination from used utensils, surfaces or equipment in all butcheries.

4.3.3 Sampling procedure

The purpose – to determine the degree of bacterial contamination of meat equipment – was communicated to the managers upon each visit, without prior notice. Most of the meat processing equipment has difficult-to-reach sites or parts, thus swabs were utilised for this purpose. Sterile cotton wool swabs which were moistened in 0.1% of peptone water prior to sampling were used. The surfaces to be sampled were rubbed for up to 30 sec over the 10 cm^2 surface area. Samples were transported to the laboratory at low temperatures of between 0°C to 4°C. Upon arrival at the laboratory, swabs were aseptically transferred to sterile McCartney bottles containing 9ml of sterile 0.1% of peptone water. Each bottle that was inoculated with the swabs, was vortexed for 30 sec to obtain a uniform mixture. Serial dilutions were prepared using 0.1% buffered peptone and inoculated in duplicate in the Plate Count Agar medium. The plates were aerobically incubated at 25°C for 48-72h (Bryan *et al*., 1997; Rajasekar and Balasubramanian, 2011). After incubation, colonies formed were counted manually and expressed as colony-forming units per square centimetre prior to their fingerprinting using MALDI-TOF MS.

4.3.4 MALDI-TOF MS fingerprinting

The Bruker Daltronics methodology was employed to identify and fingerprint microbial colonies using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF MS, Microflex™ LT benchtop, autoflex™ with BioTyper™, COMPASS™ for FLEX series incl. flexAnalysis™). 1.5 -2 ml Eppendorf tube was filled with 300 µl of ultra-pure water

(Merck, South Africa) with a pipette and 5-10 mg of the cells of the colony were transferred from the PCA plate into a 300 µl molecular grade deionised water tube. The mixture was vortexed for 30 sec, then 900 µl of (100%) absolute alcohol was added into the mixture, vortexed, and centrifuged at the maximum speed of 13,200 × g for 2 min. The supernatant was decanted and centrifuged at the same speed again. Furthermore, excess of ethanol residual was removed by pipetting carefully to avoid damaging the pellet. The pellet was air dried for 40 min at room temperature. Fifty microlitres (50 µl) of formic acid (70% v/v) was added to the pellet and vortexed for 30 sec. Subsequently, 50 µl for acetonitrile was pipetted into the mixture, vortexed carefully and centrifuged at high speed of 13,200 × g for 2 min. One microliter (1 µl) of a supernatant was spotted onto cleaned 96-spots of the stainless steel target plate (Bruker Daltronics, Germany) and the samples were allowed to air-dry at room temperature. Each dried sample on the spot of the target plate was overlaid with 1 µl of previously prepared matrix solution HCCA matrix (a saturated solution of -α cyano-4-hyrdroxy-cinnamic acid (Bruker Daltronics, Germany) in an organic solvent of a composition of 50% of acetonitrile (AN) and 2.5% of triflouroacetic acid and air-dried at room temperature to allow crystallization. An *Escherichia coli* bacterial and/or protein extract test standard (Bruker Daltronics, Germany) was used for periodic calibration of the instrument.

MALDI-TOF MS analysis of obtained isolates was executed with the Microflex LT system (Bruker Daltronics, Germany) using the manufacturer protocol. The protein ions within a range of 2000 to 20000 Da, generated with 337 nm nitrogen laser were detected in a positive linear mode fashion with the use of MALDI BioTyper automation

control. Furthermore, for the organism identification approximately 240 laser shots for each sample spot were obtained from different positions of the BTS spot using manufacturers settings. The automated species and spectra analysis was performed using the MALDI BioTyper (version 3.0). Data analysed was interpreted using manufacturers' recommendation cut-off scores of between the values of 1.7 and 1.9 was considered to be precise for genus level identification and a score of ≥2.0 demonstrated a species level. Scores with a range of 0 (no spectra) to 3.0 (perfect match) were recognized as possible outputs for the Biotyper.

4.4. Results and discussion

4.4.1 Total Viable Counts at butcheries

The Total Aerobic Viable Counts (TVC) are a conventional microbiological method that provides quantification of viable microorganisms in a sample. Traditionally, this method is done on agar plates and involves dilution of samples with results being available in approximately 24-72 h for either manual or semi-automated counts. The main objective of this study was to isolate and identify bacterial pathogens in direct and indirect meat contact surfaces from selected butcheries in the Mangaung Metropolitan Municipal area. In general, a total of 239 potential pathogenic bacterial isolates although some yeast were found and obtained from 95 samples collected at butcheries. The species mostly isolated were from the following genera: *Bacillus* (15.9%), *Pseudomonas* (12.6%), *Lactobacillus* (10%), *Staphylococcus* (8.4%), *Kocuria* (6.7%), *Acinetobacter* (4.2%), *Candid*a (2.9%), *Micrococcus* (2.9%), *Escherichia coli* (1.7%), *Neisseria* (1.7%),

amongst others (illustrated in 4.4.2 below). The total bacterial counts on surfaces at the butcheries ranged from 3.24×10^3 cfu.cm^{-2} to 6.57×10^3 cfu.cm^{-2} as shown in Figures 4.1 to 4.5.

The average counts at butchery A were between 1.56×10^1 cfu.cm^{-2} and 1.79×10^2 cfu.cm^{-2} with a group data standard deviation of STD_g= 42.1 were observed on the trays and floors respectively. The highest microbial load of 2.44×10^2 cfu.cm^{-2} was found on the floor surfaces, which may be attributed to the worn out or cracked floors providing environments conducive to the hiding and breeding of microorganisms as well as hindering the effectiveness of cleaning procedures (Sudhakar *et al.,* 2009; Ali *et al.,* 2010). Tables in butchery A were observed to be dirty when visually inspected; however, no microbial loads were observed (Figure 4.1).

In butchery B, the group data standard deviation of STD_g= 36.5 with was recorded with the average counts between 2.48×10^1 cfu.cm^{-2} (tables) and 1.96×10^2 cfu.cm^{-2} (floors). The highest counts were observed on floor surfaces at a level of 2.17×10^2 cfu.cm^{-2} which may be attributed to the sawdust used on floors to make them less slippery (Figure 4.2). Moreover, the use of this sawdust may result in failure of effective cleaning and sanitation of floors, harbouring bacteria that can have serious health implications as well as shortening the shelf-life of meat and its products (Verran *et al.,* 2008; Koo *et al*., 2013a; Koo *et al*., 2013b). No microbial loads were observed on tables, scale display, bowl cutter, or meat slicer surfaces. All of the above-mentioned

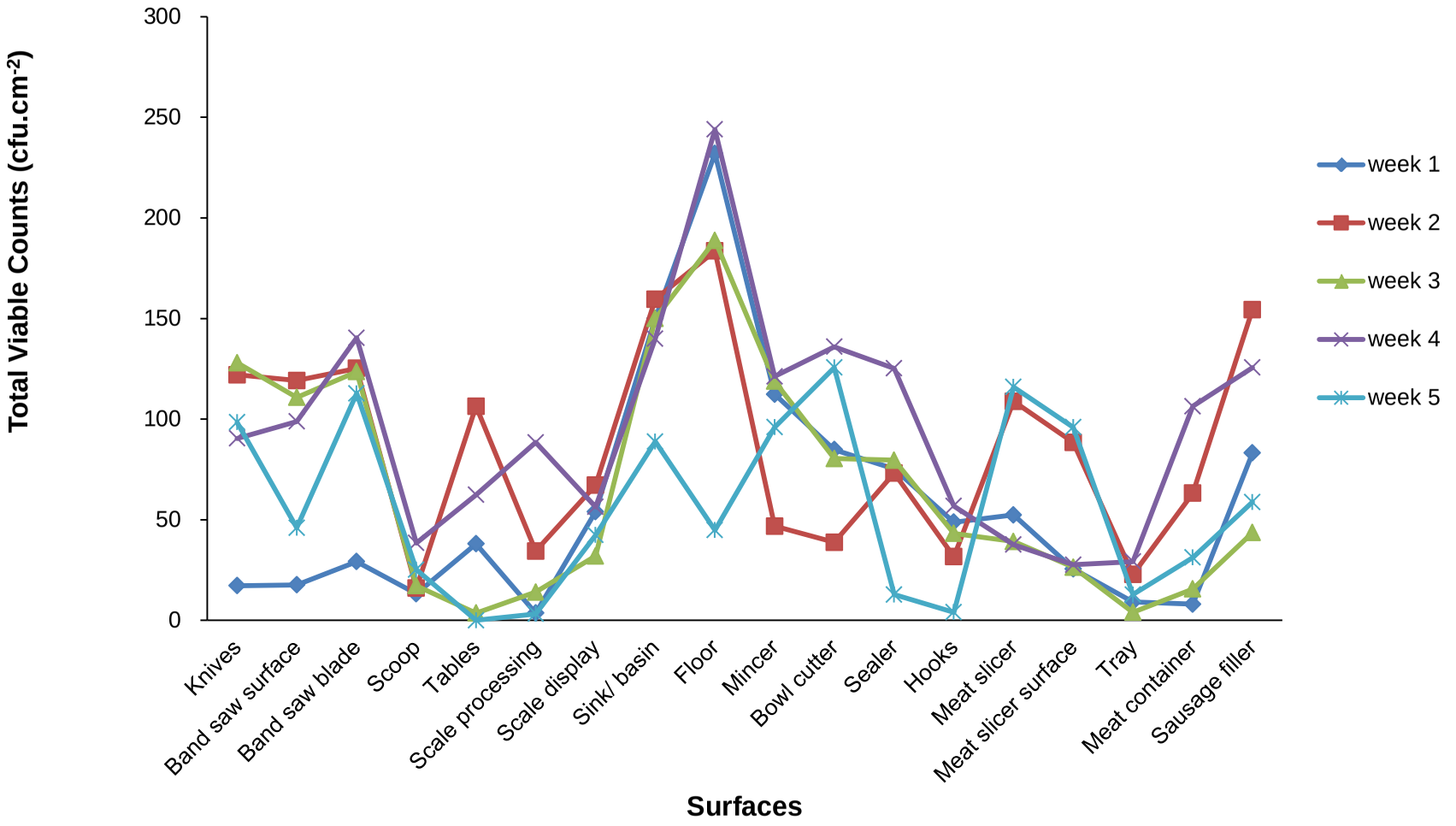

Figure 4.1: Total Viable Counts on environmental surfaces in butchery A

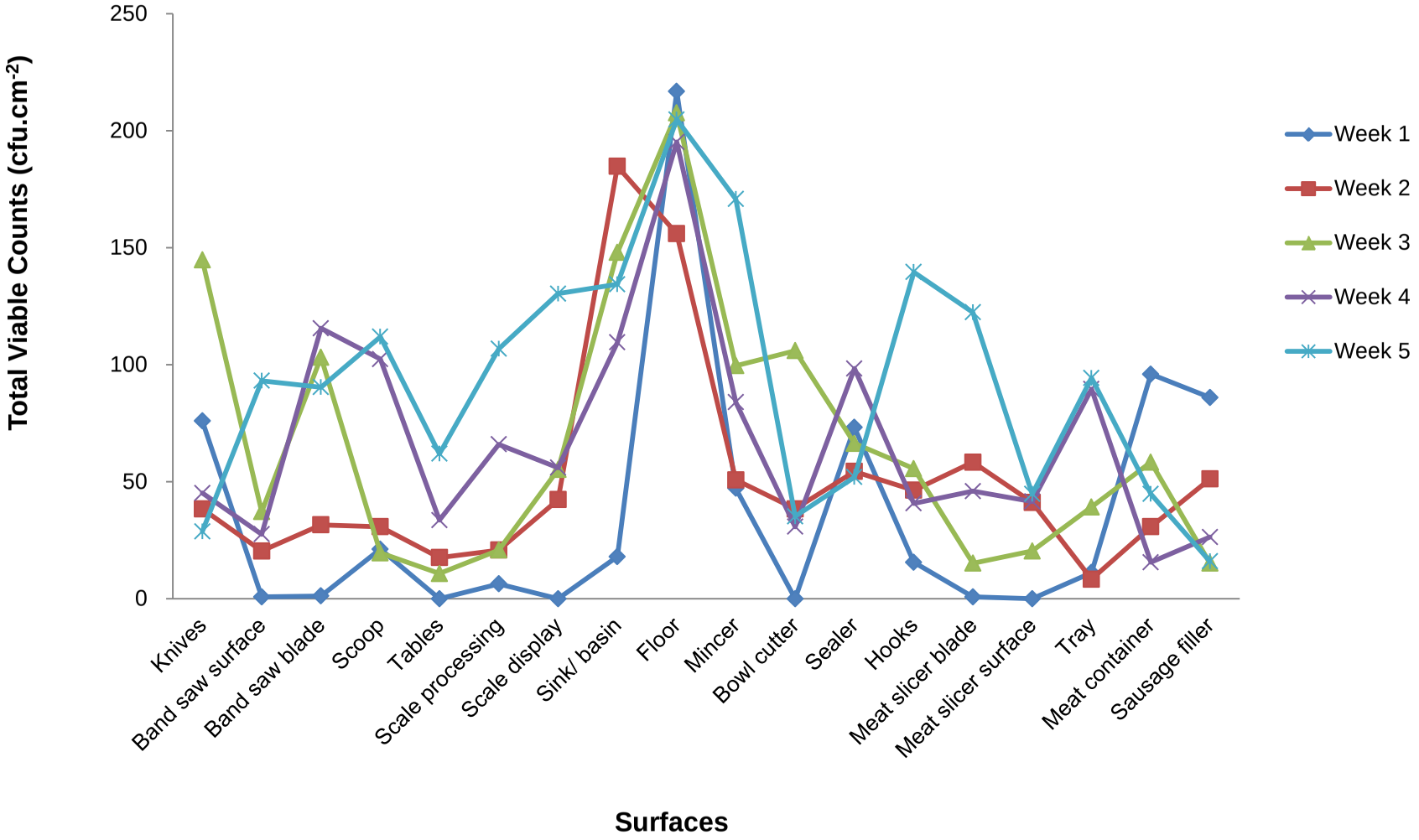

Figure 4.2: Total Viable Counts on environmental surfaces in butchery B

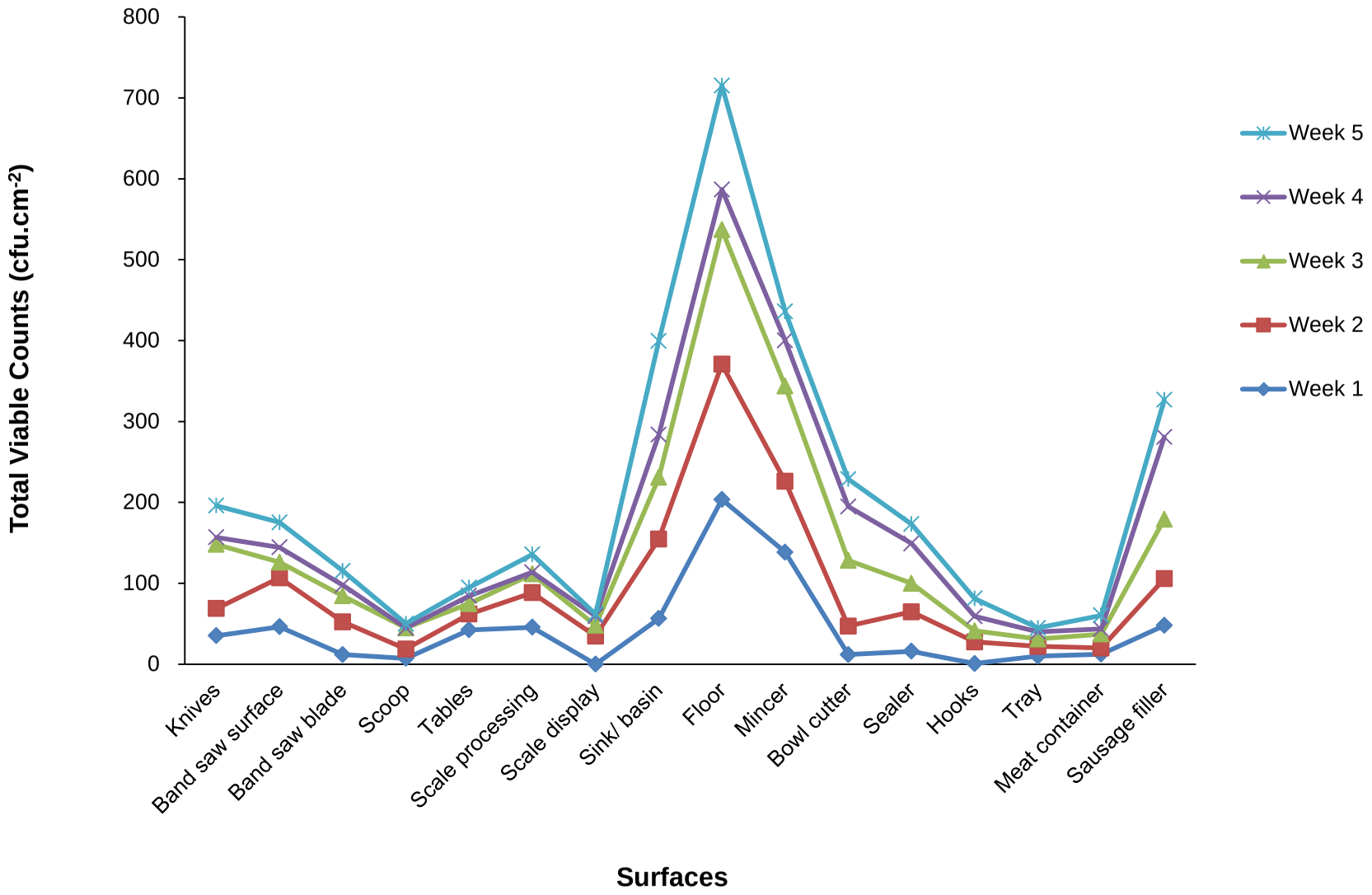

Figure 4.3: Total Viable Counts on environmental surfaces in butchery C

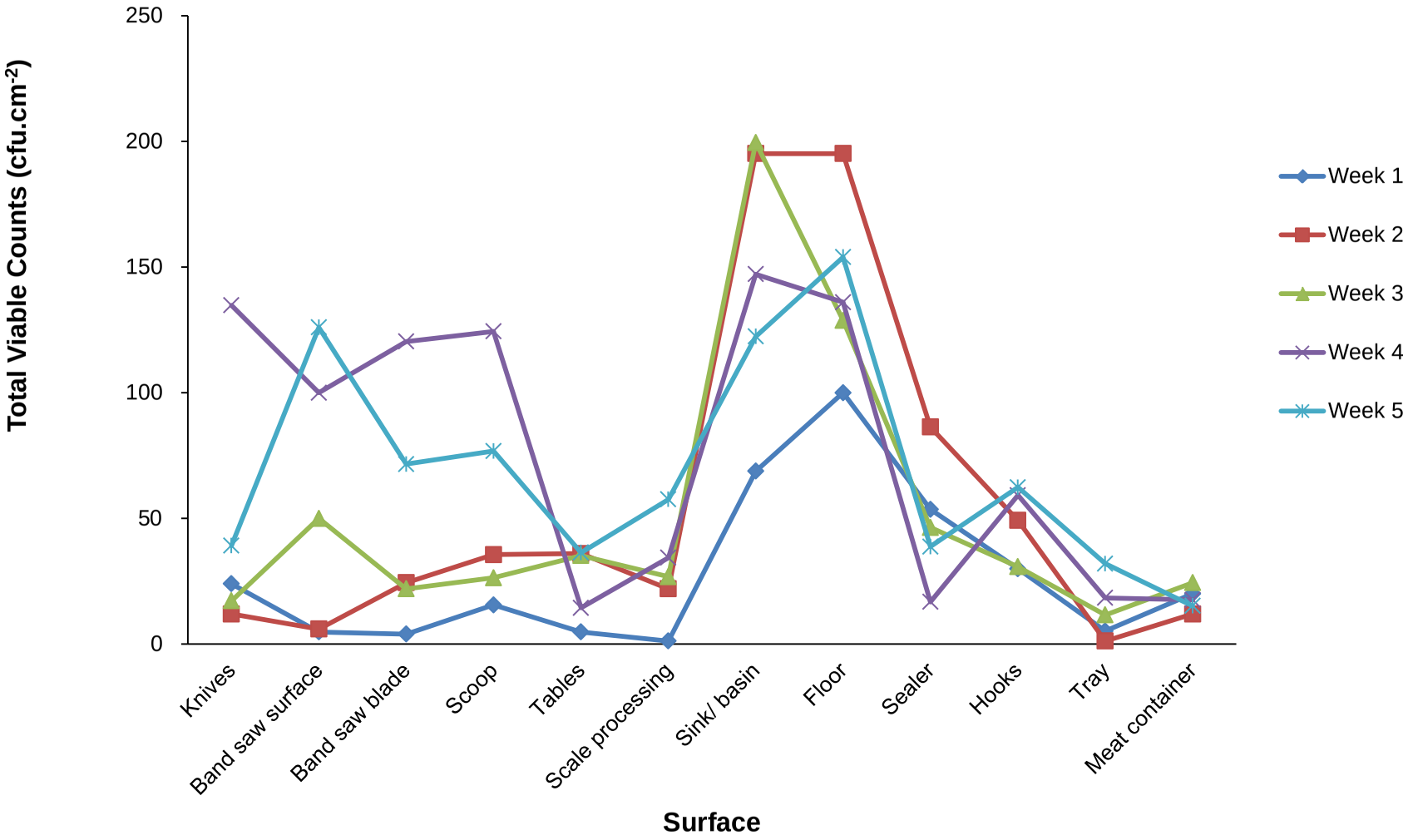

Figure 4.4: Total Viable Counts on environmental surfaces in butchery D

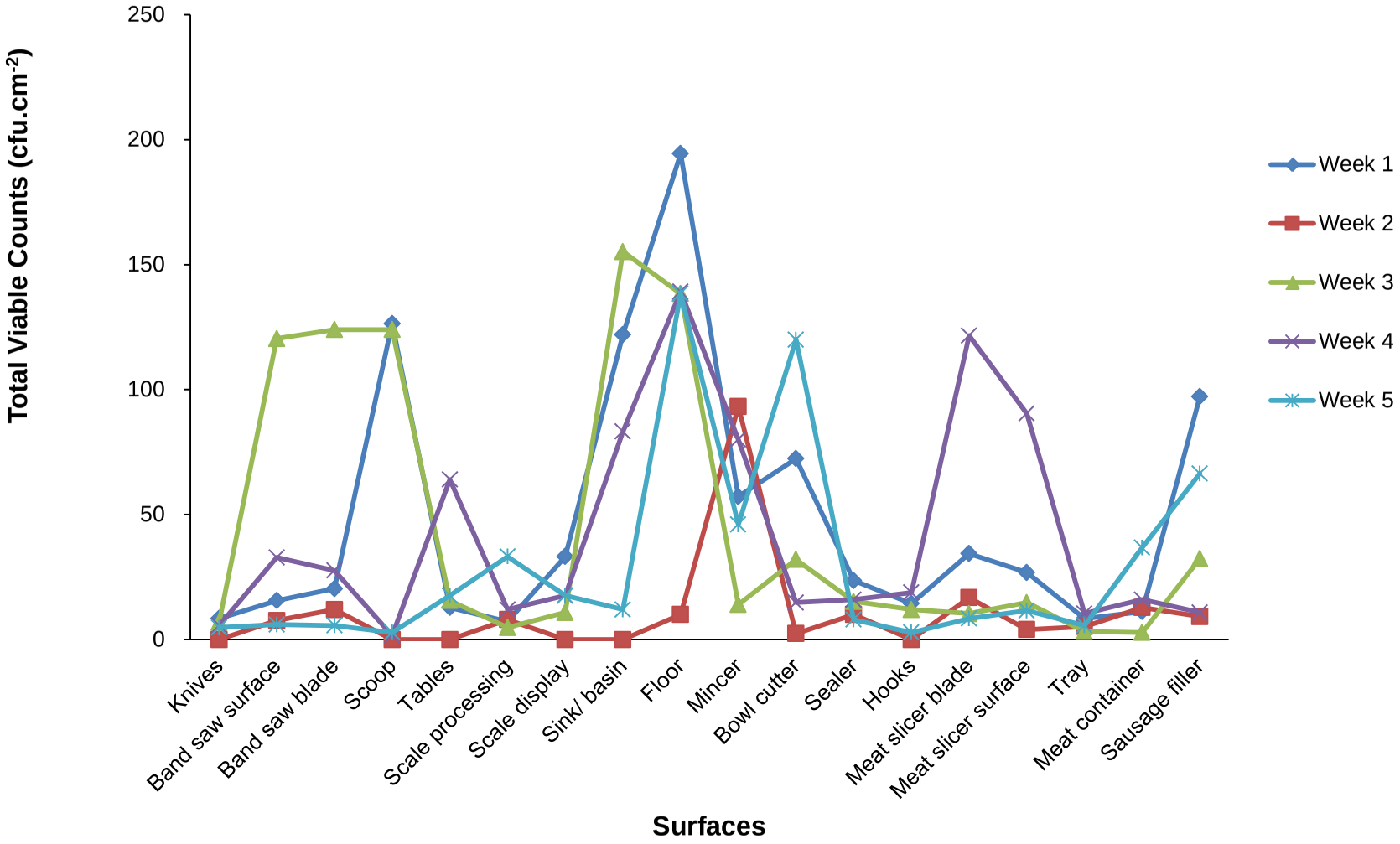

Figure 4.5: Total Viable Counts on environmental surfaces in butchery D

equipment is made from stainless steel that is smooth, non-porous and therefore easy to clean, reducing the ability of microbes to breed on them with proper cleaning and sanitization.

Butchery C had average counts ranging from 8.9×10^0 cfu.cm^{-2} to 1.43×10^2 cfu.cm^{-2}. Floors had the highest bacterial levels of 2.04×10^2 cfu.cm^{-2} whilst no counts were observed on surfaces of scoops and scale in the display area (Figure 4.3). Although the level of hygiene in butchery C was good, the layout of the butchery may have contributed to the high bacterial levels as a result of the activities involved in the area. Plastic containers were used to package meat products prior to weighing and these could be the reason why no microbial counts were observed on the scale in the display area. In addition, Table 4.1 below reflect significant differences between butcheries over the entire sampling period with regard to total viable counts quantified from surfaces and working utensils.

The average counts in butchery D were between 1.37×10^1 cfu.cm^{-2} and 1.47×10^2 cfu.cm^{-2}, whilst the highest and lowest occurrences were observed at levels of 2×10^2 cfu.cm^{-2} (sink surface) and 1.20×10^0 cfu.cm^{-2} (tray and scale surfaces) respectively (Figure 4.4). This observation was different from other butcheries in which high and low counts were mostly observed on floor and scoop surfaces; however, the findings were in agreement with those of Chmielewski and Frank (2003) who found high microbial levels on damp surfaces. Additionally in this study, high counts could be attributed to the

Table: 4.1: Significant values for TVC counts within selected butcheries.

Butcheries grouping	**Significant value (p)**
A and B	0.83
A and C	0.59
A and D	0.15
A and E	0.86
B and C	0.74
B and D	0.22
B and E	0.71
C and D	0.39
C and E	0.51
D and E	0.14

use of only one sink for all the meat processing activities including hand-washing. With a lot of processing activities, the settings of the butchery and high consumer movement/traffic, the findings at this butchery were interesting as the bacterial levels were generally lower than anticipated which was in contrast to what Rahkio and Korkeala (1997) reported in their study.

At butchery E (Figure 4.5), the average levels ranged between 5.12×10^{0} cfu.cm^{-2} and 1.24×10^{2} cfu.cm^{-2}, with the highest counts being 1.94×10^{2} cfu.cm^{-2} (floors). No microbial levels were observed on surfaces which include knives, scoops, scales, sinks, and hooks. Generally the counts from the floor surface in butchery C were the lowest compared to the other butcheries (A, B, D and E), which could be due to the layout of the butchery that provides sufficient room for movement of employees, flooring material that is conducive to easy and thorough cleaning as well as good hygiene practices (Rahkio and Korkeala, 1997).

4.4.2 Isolated microorganisms

Rapid identification of pathogenic microorganisms in food processing environments is a requirement in the effort to ensure a safe food supply. Foodborne pathogens spread easily and fast due to several reasons, namely: (1) food products such as meat are highly perishable and produce a suitable medium for growth of microbes. Thus, sanitation on meat contact surfaces is of outmost importance for food safety and quality; (2) the designs of utensils and equipment can potentially pose a risk of contamination to

food depending on their cleaning accessibility and hygiene status; and (3) uncontrolled movement of food handlers from dirty areas to clean areas. The predominant reason for meat contamination leading to spoilage in butcheries is ineffective methods of cleaning. Moreover, when there is a lack of environmental monitoring, the efficacy of chemical disinfectants, cleaning techniques and microbial loads cannot be recognized and monitored. Studies indicate that nonporous food contact surfaces such as table tops, meat slicers, bowl cutters and scales can harbour bacterial pathogens with low infectious dose, which can survive for a few weeks on surfaces (Flores, 2006).

4.4.2.1 Gram-positive bacterial isolates

***Bacillus* spp.**

Members of the genus *Bacillus* are Gram-positive, aerobic, spore-forming rods that are ubiquitous in nature. They are largest *Bacillaceae* group encompassing more than 60 species (Priest, 1993). Food poisoning bacilli are distributed in foods such as meat and meat products, milk-based products and soups which are associated with diarrhoeal syndrome. A foodborne pathogen *Bacillus cereus* was reported in 1906, to be the cause of an outbreak involving 300 patients after the consumption of contaminated meat balls.

***Staphylococcus* spp.**

Staphylococcus genus is ubiquitous in nature and a number of species were isolated in the current study (Table 4.2). The high prevalence of *Staphylococcus* isolates suggests

Table 4.2: Microbial profile isolated from environmental samples at butcheries

Items	Strains isolated	Butcheries				
		A	B	C	D	E
Knives	*Bacillus subtilis* ssp. *subtilis* DSM 10T DSM					X
	Bacillus subtilis ssp. *subtilis* DSM 5660 DSM				X	X
	Candida lusitaniae[ana] (Clavispora lusitaniae) CBS 4413T CBS					X
	Kocuria rhizophila DSM 348 DSM					X
	Kocuria kristinae IBS		X			
	Kocuria rhizophila DSM 348 DSM		X			
	Lactobacillus reuteri DSM 17938			X		X
	Lactobacillus vitulinus DSM 20405T DSM					X
	Microbacterium oxydans DSM 20578T DSM					X
	Moraxella sg Moraxella osloensis DSM 6359 DSM	X				
	Pseudomonas oleovorans DSM 1045T HAM			X		
	Pseudomonas putida DSM 291T HAM					X
	Staphylococcus aureus ATCC 33591 THL	X				
	Staphylococcus capitis ssp. *urealyticus* DSM 6717T DSM		X			
	Staphylococcus pasteuri DSM 10656T DSM	X				
Bandsaw surface	*Arthrobacter arilaitensis* DSM 16368T DSM					X
	Bacillus subtilis ssp. *subtilis* DSM 10T DSM				X	X
	Bacteroides suis DSM 20612T DSM					X
	Candida lusitaniae[ana] (Clavispora lusitaniae) CBS 4413T CBS				X	
	Candida sorbosa[ana] (Issatchenkia occidentalis) CBS 1910 CBS					X
	Citrobacter braakii 93142 CHB			X		
	Hafnia alvei M110266 LDW			X		
	Kocuria kristinae N235M19QSA IBS					X
	Kocuria rhizophila DSM 11926T DSM			X		X
	Kytococcus sedentarius IMET 11362T HKJ			X		
	Lactobacillus kimchii DSM 13961T DSM	X				
	Macrococcus caseolyticus DSM 20597T DSM					X
	Micrococcus luteus 59 PIM					X
	Serratia liquefaciens CCM 2716 CCM	X	X			
	Sphingomonas panni DSM 15761T DSM					X
	Staphylococcus saprophyticus ssp. *bovis* DSM 18669T DSM					X
	Staphylococcus saprophyticus ssp. *saprophyticus* CCM 2682 CCM	X				
	Staphylococcus vitulinus DSM 15615T DSM					X
	Thaueraterpenica 58Eu MPB	X				
	Trichophyton rubrum VML	X				
Bandsaw blade	*Aeromonas eucrenophila* CECT 4224T DSM				X	
	Bacillus cereus DSM 31T DSM					X
	Bacillus endophyticus DSM 13796T DSM					X
	Bacillus subtilis ssp. *subtilis* DSM 10T DSM					X
	Lactobacillus kimchii DSM 13961T DSM	X				
	Micrococcus luteus IMET 11249 HKJ				X	
	Ochrobactrum tritici DSM 13340T HAM		X			
	Pseudomonas extremorientalis DSM 15824T HAM					X
	Pseudomonas fulva 013W30 NFI				X	

	Pseudomonas koreensis LMG 21318T HAM					X
	Pseudomonas lundensis DSM 6252T HAM					X
	Pseudomonas stutzeri B367 UFL				X	
	Pseudomonas tolaasii LMG 2342T HAM					X
	Ralstonia pickettii 213231 CHB			X		
	Serratia liquefaciens CCM 2716 CCM	X				
	Staphylococcus saprophyticus ssp. *bovis* DSM 18669T DSM					X
	Staphylococcus saprophyticus ssp. *saprophyticus* CCM 2682 CCM	X	X			X
	Staphylococcus vitulinus DSM 15615T DSM				X	
	Staphylococcus xylosus DSM 6179 DSM		X			
	Thaueraterpenica 58Eu MPB	X				
	Trichophyton rubrum VML	X				
	Trichosporon mucoides ATCC 204094 THL				X	
Scoop	*Arthrobacter pyridinolis* B384 UFL				X	
	Bacillus amyloliquefaciens CIP 103265T CIP					X
	Bacillus licheniformis CS 541 BRB		X			
	Bacillus pumilus DSM 1794 DSM					X
	Bacillus subtilis ssp. *subtilis* DSM 10T DSM				X	X
	Candida lusitaniae[ana] (Clavispora lusitaniae) CBS 4413T CBS				X	X
	Kocuria rosea IMET 11363T HKJ					X
	Macrococcus caseolyticus DSM 20597T DSM				X	
	Moraxella sg Moraxella osloensis 76 PIM	X				
	Pseudomonas aeruginosa ATCC 27853 THL		X			
	Pseudomonas fragii DSM 3456T HAM		X	X		X
	Pseudomonas koreensis LMG 21318T HAM ISOLATED					X
	Staphylococcus sciuri ssp. *sciuri* DSM 6671 DSM				X	
Tray	*Acinetobacter haemolyticus* LMG 1033 HAM					X
	Arthrobacter aurescens DSM 20116T DSM					X
	Bacillus pumilus DSM 1794 DSM					X
	Kocuria carniphila DSM 16004T DSM					X
	Pseudomonas chlororaphis ssp. *chlororaphis* DSM 50083T HAM					X
	Ralstonia pickettii 213231 CHB		X			
	Raoultella terrigena DSM 7331 DSM					X
Tables	*Acinetobacter haemolyticus* LMG 1033 HAM					X
	Arthrobacter aurescens DSM 20116T DSM					X
	Bacillus licheniformis 992000432 LBK	X			X	
	Bacillus pumilus DSM 1794 DSM				X	X
	Citrobacter braakii 9314_2 CHB		X			
	Kocuria carniphila DSM 16004T DSM					X
	Kocuria rhizophila DSM 348 DSM	X				
	Lactobacillus delbrueckii ssp. *lactis* DSM 076 DSM	X				
	Micrococcus luteus IMET 11249 HKJ				X	
	Pseudomonas chlororaphis ssp. *chlororaphis* DSM 50083T HAM					X
	Pseudomonas lundensis DSM 6252T HAM			X		
	Raoultella terrigena DSM 7333					X
	Yersinia enterocolitica ssp. *enterocolitica* (serovar O8) ATCC 9610T THL		X			
Scale proce	*Arthrobacter oxydans* IMET 10684T HKJ					X
	Bacillus pumilus DSM 1794 DSM					X

	Bacillus subtilis ssp. *subtilis* DSM 10T DSM				X	
	Chryseobacterium indologenes CCM 4451T CCM					X
	Clostrdium bifermentans 2274CCUG 35556 A BOG	X				
	Kocuria rhizophila DSM 348 DSM		X			
	Lactobacillus plantarum DSM 12028 DSM	X				
	Ochrobactrum intermedium LMG 3301T HAM	X				
	Pseudomonas koreensis 22 TUB					X
	Pseudomonas lundensis DSM 6252T HAM		X			
	Raoultella terrigena DSM 7333 DSM i					X
	Serratia marcescens 131031CHB					X
Scale display	*Arthrobacter creatinolyticus* DSM 15881T DSM				X	X
	Bacillus atrophaeus DSM 2277 DSM					X
	Bacillus licheniformis CS 541 BRB	X				
	Citrobacter freundii 220541 CHB			X		
	Kocuria rhizophila DSM 11926T DSM	X				
	Lactobacillus curvatus DSM 20010 DSM		X			
	Lactobacillus reuteri DSM 17938					X
	Staphylococcus vitulinus DSM 15615T DSM					X
Sink/Basin	*Aureobasidium pullulans* 15131 CBS		X			
	Acinetobacter haemolyticus LMG 1033 HAM				X	X
	Acinetobacter junii DSM 6964T HAM			X		
	Aeromonas hydrophila ssp. *anaerogenes* DSM 30188T HAM				X	
	Bacillus amyloliquefaciens CIP 103265T CIP				X	
	Bacillus pumilus DSM 1794 DSM					X
	Bacillus safensis CIP 109412 CIP					X
	Bacillus subtilis DSM 5611 DSM					X
	Candida lambica[ana] (Pichia fermentans ssp *fermentans)* CBS 603 CBS				X	
	Enterococcus faecium 11037 CHB			X		
	Escherichia coli DH5alpha BRL				X	
	Kocuria rhizophila DSM 11926T DSM		X	X	X	
	Lactobacillus fructivorans DSM 20203T DSM				X	
	Micrococcus luteus IMET 11249 HKJ				X	
	Moraxella sg Moraxella osloensis DSM 6359 DSM	X				
	Pseudomonas fragii DSM 3456T HAM	X				
	Pseudomonas mendocina DSM 50017T HAM				X	
	Pseudomonas stutzeri DSM 5190T HAM					X
	Rothia dentocariosa G6496ch28 IBS			X		
	Rothia nasimurium 10036873108 USH			X		
	Serratia liquefaciens DSM 30125 DSM	X				
	Streptococcus salivarius 0807M25049501 IBS			X		
Floors	*Aureobasidium pullulans* 15131 CBS				X	
	Acinetobacter baumannii B389 UFL	X	X			
	Acinetobacter haemolyticus LMG 1033 HAM				X	X
	Bacillus ruris DSM 17057T DSM					X
	Candida lusitaniae[ana] (Clavispora lusitaniae) CBS 4413T CBS					X
	Corynebacterium xerosis DSM 20743T DSM					X
	Enterococcus faecalis 202474 CHB	X				
	Lactobacillus gastricus DSM 16046 DSM					X

	Lactobacillus nagelii DSM 13675T DSM		X			
	Legionella moravica DSM 19234T DSM					X
	Neisseria meningitides Serogroup Y BRL	X				
	Pseudomonas aeruginosa ATCC 27853 THL		X			
	Sphingobacterium faecium DSM 11690T HAM			X		
Mincer	*Acinetobacter johnsonii* DSM 6963T					X
	Aeromonas bestiarum CECT 4227T DSM			X		
	Bacillus cereus 4080 LBK		X			X
	Bacillus subtilis ssp. *subtilis* DSM 5660 DSM					X
	Campylobacter jejuni MB611105 THL					X
	Corynebacterium callunae DSM 20147T DSM					X
	Lactobacillus fuchuensis DSM 14341 DSM					X
	Microbacterium oxydans DSM 20578T DSM					X
	Pseudomonas lundensis DSM 6252T HAM		X			
	Pseudomonas stutzeri DSM 5190T HAM					X
	Staphylococcus equorum ssp. *equorum* DSM 20675 DSM			X		
	Staphylococcus saprophyticus ssp. *bovis* DSM 18669T	X				
	Staphylococcus xylosus DSM 6179 DSM		X			
	Tissierella praeacuta 1078 ATCC 33268T BOG		X			
Hooks	*Arthrobacter sulfureus* DSM 20167T DSM					X
	Bacillus megaterium DSM 32T DSM					X
	Bacillus subtilis ssp. *subtilis* DSM 5660 DSM)					X
	Candida lusitaniae[ana] (*Clavispora lusitaniae*) CBS 4413T CBS					X
	Citrobacter freundii 131582 CHBx2			X		
	Lactobacillus agilis DSM 20508 DSM					X
	Lactococcus garvieae DSM 20684T DSM	X				
	Pseudomonas koreensis LMG 21318T HAM					X
	Pseudomonas pictorum LMG 981T HAM			X		
	Rothia nasimurium 10036873108 USH			X		
	Staphylococcus aureus ssp. *anaerobius* DSM 20714	X				
	Staphylococcus epidermidis 10547 CHB	X				
	Weissella halotolerans DSM 20190T DSM			X		
Sealer	*Acinetobacter baumannii* B389 UFL		X			
	Acinetobacter lwoffii 2Ring240 MHH		X			
	Bacillus licheniformis CS 541 BRB		X			X
	Bacillus subtilis ssp. *subtilis* DSM 10T DSM					X
	Citrobacter freundii 220541 CHB			X		
	Kocuria rhizophila DSM 348 DSM		X			
	Lactobacillus plantarum DSM 20205 DSM					X
	Lactobacillus saerimneri DSM 16049T DSM					X
	Micrococcus luteus N203 CPB	X	X		X	
	Moraxella sg Moraxella osloensis 76 PIM	X				
	Neisseria flavescens C1 2 PGM			X		
	Pseudomonas fragii DSM 3456T HAM		X			
	Rothia dentocariosa G6496ch28 IBS			X		
	Staphylococcus lugdunensis DSM 4804T DSM				X	
Meat slicer	*Arthrobacter oxydans* DSM 20119T DSM					X
	Bacillus pumilus DSM 1794 DSM					X

	Gordonia rubripertincta DSM 43303 DSM	X				
	Kocuria rhizophila DSM 46222 DSM	X				
	Lactobacillus sakei ssp. *sakei* DSM 20017T DSM	X				
	Microbacterium oxydans DSM 20578T DSM					X
	Micrococcus luteus N203 CPB	X				
	Pseudomonas koreensis 22 TUB					X
	Pseudomonas stutzeri B367 UFL					X
Meat slicer surface	*Bacillus amyloliquefaciens* CIP 103265T CIP					X
	Kocuria rhizophila DSM 348 DSM x2	X				
	Lactobacillus agilis DSM 20510 DSM					X
	Lactobacillus ruminis DSM 20404 DSM					X
	Lactobacillus sharpeae DSM 20506 DSM	X				
	Macrococcus caseolyticus DSM 20597T DSM	X				
	Pseudomonas koreensis LMG 21318T HAM					X
	Pseudomonas stutzeri DSM 5190T HAM					X
Tray	*Aureobasidium pullulans* 16419 CBS	X				
	Bacillus licheniformis CS 541 BRB	X				
	Bacillus safensis CIP 109412 CIP					X
	Enterobacter cloacae DSM 30060 DSM				X	
	Lactobacillus equi DSM 15833T DSM					X
	Micrococcus luteus IMET 11249 HKJ			X	X	
	Neisseria meningitides Serogroup Y BRL	X				
	Pseudomonas koreensis 22 TUB I					X
	Rothia aeria DSM 14556T DSM			X		
Sausage filler	*Acinetobacter lwoffii* DSM 2403T DSM	X				
	Bacillus amyloliquefaciens CIP 103265T CIP					X
	Bacillus cereus 994000168 LBK					X
	Bacillus subtilis ssp. *subtilis* DSM 5660 DSM					X
	Candida lusitaniae[ana] (Clavispora lusitaniae) CBS 4413T CBS					X
	Corynebacterium variabile DSM 44702 DSM					X
	Escherichia coli ATCC 25922 THL	X				
	Escherichia coli DH5alpha BRL		X			
	Lactobacillus reuteri DSM 17938			X		
Meat container	*Arthrobacter monumenti* DSM 16405T DSM	X				
	Arthrobacter nasiphocae DSM 13988T DSM	X				
	Bacillus endophyticus DSM 13796T DSM					X
	Kocuria rhizophila DSM 46222 DSM	X				
	Lactobacillus kimchii DSM 13961T DSM	X				
	Micrococcus luteus BK0114009 ERL	X				
	Neisseria meningitides Serogroup Y BRL			X		
	Paracoccus versutus B352 UFL	X				
	Rhodotorula mucilaginosa DSM 70403 DSM				X	X
Bowl cutter	*Bacillus amyloliquefaciens* CIP 103265T CIP					X
	Bacillus subtilis ssp. *subtilis* DSM 5660 DSM					X
	Escherichia coli ATCC 35218 CHB	X				
	Lactobacillus sakei ssp. *sakei* DSM 20017T DSM					X
	Micrococcus luteus IMET 11249 HKJ					X
	Staphylococcus xylosus DSM 6179 DSM		X			
	Streptomyces lavendulae B264 UFL		X			

poor personal and general hygiene practices at the butcheries (Tong *et al.,* 2011). Food handlers with respiratory infections may have also played a role in the transmission and dispersal of these organisms to the meat contact surfaces by sneezing and coughing. Thus, it is important that workers be encouraged to report their illnesses, as well as any fresh wounds from knife or band saw cuts. Meat is handled frequently during processing hence it is regarded as a prime target for staphylococci contamination. Several studies have shown that *Staphylococcus* species have the ability to colonize surface materials as a form of survival in the natural environment and that they resist cleaning and disinfection (Bagge-Ravn *et al*., 2003; Kusumanigrum *et al*., 2003).

In the current study, *Staphylococcus* coagulase positive *S. aureus* and coagulase negative *S. saprophyticus* were isolated from various items in butchery A, B and E and their presence is a concern as they are known to be causative agents of food poisoning. However, strains of *S. xylosus* and *S. carnosus,* which are considered the most important staphylococcal species in the meat industry as they are used as starter cultures for fermented sausages, were also isolated in the current study (Corbière Morot-Bizot *et al.,* 2007).

***Lactobacillus* spp.**

The genus *Lactobacillus* is the most important and diverse group amidst *Lactobacillaceae* family which includes over 100 known species that are applied as preservatives in the production of functional foods (Satokari *et al*., 2003; Sanders,

2003). These Gram-positive lactobacilli have a significant and wide usage in the pharmaceutical industry and in the production of food necessitating lactic acid fermentation, particularly fermented meats (salami), fermented vegetables (pickles) and dairy products (yogurt). In the current study a well-established probiotic strain of *Lactobacillus reuteri* was isolated from knives in two of the butcheries. Several studies have comprehensively characterized *L. reuteri* DMS 17938 for their probiotic properties, occurring naturally in the human gastrointestinal tract (Savino *et al*., 2007; Rosander *et al*., 2008; Dommels *et al*., 2009; Dimaguila *et al*., 2013). Probiotics are living microorganisms which upon adequate administration or ingestion confer health benefits upon the host (FAO, 2002). In the meat industry, the reuterin produced from the *Lactobacillus reuterin* strain is used for meat decontamination and preservation (Ruiz-Moyano *et al*., 2009). Accordingly, its isolation in this study on the tables, in sausage fillers and in bowl cutters in butcheries C and E was understandable.

***Micrococcus* spp.**

Micrococcus are non-sporulating, Gram-positive bacteria, commonly isolated from the skin and surfaces of inanimate objects. Thus it was reasonable in this study for predominance of this species to be found in the butcheries (Table 4.2). *Micrococcus luteus,* which dominates amongst the human skin flora (Kloos *et al.,* 1998), was isolated from the surfaces at butcheries. In a study conducted by Królasik *et al.* (2010), among microorganisms exhibiting resistance to disinfectant after disinfecting food contact surfaces, *M. luteus* showed the lowest resistance compared to other bacterial species.

In the meat industry, it has previously been isolated in raw-cured meat products, and laćon may be associated with handling.

4.4.2.2 Gram-negative bacterial isolates

Enterobacteriaceae are the most investigated organism, often used as the indicators of faecal contamination. The most prevalent *Enterobacteriaceae* isolated in this study included *E. coli, Klebsiella, Enterobacter, Serratia, Citrobacter, Hafnia* and *Morganella*. The presence of *E. coli* on meat contact surfaces is not uncommon as it is predominant in the gastrointestinal tract of humans and warm blooded animals. *Enterobacteriaceae* presence reveals faecal contamination and poor sanitary practices during meat processing (Habimana *et al*., 2010). Infections of some strains of *E. coli* can be harmless while others can cause disease in human beings, ranging from bloody diarrhoea, haemolytic ureamic syndrome to fatality (Buchanan and Doyle, 1997; Johnson *et al.,* 2002). In 2005, outbreaks of *E. coli* 0157 infections and deaths were associated with the food hygiene failures at the butchery premises (Pennington, 2009). It is for such reasons that meat processors must be encouraged to reduce the cause of such incidences by optimizing meat hygiene practices.

***Serratia* spp.**

Serratia liquefaciens was one of the major isolates in butcheries A and B, followed by *Hafnia alvei* in butchery C both of which are known to be causative spoilage agents in

chilled meat and also known to cause infection in fish. Additionally, the isolation of the aforementioned species may be used as indicative of effective cleaning and sanitation. The results are similar and in agreement with studies done by Rahkio and Korkeala (1997), Olsson *et al*. (2003) and Guðbjörnsdóttir *et al*. (2005).

Enterococci spp.

Enterococci were largely isolated in butchery D particularly in trays. These organisms are widely distributed in nature and under normal conditions they are harmless. In immune-compromised patients, however, they become opportunistic pathogens with the capability of causing nosocomial bloodstream infections (Mezzatesta *et al*., 2012). Other *Enterobacteriaceae* identified in this study were *Citrobacter braakii, Citrobacter freundii, Yersinia enterolitica* in butcheries B and C. The latter isolated species is of major concern in the food industry because they can grow well at refrigeration temperature and are capable of causing serious illness in humans ranging from self-limiting gastroenteritis to death (Letellier *et al*., 1999; Grahek-Ogden *et al*., 2007). Moreover, its incidence roughly rivals that of *Salmonella* as a food pathogen. Although they are widely isolated from a variety of foods, pork remains the significant reservoir of this pathogen. In this study the *Y. enterocolitica* strain was isolated from tables and meat mincer equipment. In a similar study, Vishnubhatla *et al*. (2001) reports a high occurrence of *Y. enterocolitica* in minced meat. *Yersina enterocolitia* with other three species (*Yersinia kristensenii*, *Yersinia frederiksenii*, and *Yersinia intermedia)* have been widely observed as food contaminants. Therefore it is reasonable to conjecture that unclean mincers and

other meat processing equipment are possible transmission vehicles of yersiniosis which is a zoonotic pathogen.

Acinetobacter spp.

The genus *Acinetobacter* and its members emerged as a significant nosocomial pathogen. They are Gram-negative, isolated mostly from fresh produce foods with spoilage implications in foods such as bacon, chicken, fish, eggs and hospital foods. This spoilage bacterium is common in chilled and vacuumed packed meat (Dainty and Mackey, 1992). The occurrence of both *Acinetobacter baummannii* and *Acinetobacter haemolyticus* (Table 4.2) in the butchery environments is a cause for a concern in this study due to their remarkable ability to cause hospital-acquired infections. Complete eradication of both endemic *Acinetobacter* species is not easy because of their resistance to antibiotics (Doughari *et al.*, 2012).

Pseudomonas spp.

Pseudomonas spp. are the most important spoilage bacteria and their high representation in Table 4.2 could be due to their ubiquitous distribution in soil, water and animals. Their ability to spoil meat under aerobic chilled storage conditions is well documented (Jay *et al*., 2003; Ercolini *et al*., 2006). In this study, *Pseudomonas* species were isolated from environmental surfaces such as hooks, knives, band saw blade, scoops and tables amongst others (Table 4.2). The dominance of *Pseudomonas*

species in this study indicated the extensive environmental contamination found in butcheries.

Pseudomonas species isolated in this study are usually characterized as either food contaminants or surface-colonizing strains. *Pseudomonas fragii* has the ability to adhere to stainless steel surfaces in food processing establishments, resulting in the formation of biofilm communities. Similarly, *P. fragii* was predominantly isolated on meat processing equipment made of stainless steel at butcheries (A, B, C and E). The isolation of *Pseudomonas lundensis* was a concern as this species is known to cause meat spoilage and can proliferate at low temperatures (Gennari and Dragotto, 1992; Doyle, 2007). *Pseudomonas aeruginosa* is widely distributed in nature, and in the current study it was isolated from floors and scoops (Cheesbrough, 2000). The presence of *Pseudomonas aeruginosa* on aforementioned surfaces can be detrimental to the microbial status of meat processed at the sampled butcheries. *Pseudomonas aeruginosa* has been reported as an opportunistic bacterial pathogen, hazardous particularly to immune-compromised persons; however, the ingestion of *P. aeruginosa* rarely causes foodborne illnesses (Cheesbrough 2000; Lister *et al.,* 2009).

Neisseria spp.

The fact that *Neisseria meningitis* was isolated from butcheries A and C came as surprise and was a major concern in this study. Members of the genus *Neisseria* are Gram-negative and are normal inhabitants of mucous membrane surfaces which

include the upper respiratory tract of human beings (Rouphael and Stephens, 2012). These pathogenic bacteria are causative agents of an array of infectious diseases ranging from occult sepsis with rapid recovery to fulminant overwhelming fatal diseases. Transmission of *Neisseria meningitis* is normally achieved through direct contact with a person who has the infection, through droplets or secretions from the upper respiratory tract. The isolation of *Neisseria meningitis* in butcheries A and C has been a concern as these could pose a significant public health risk to meat consumers. A study conducted by Jorgensen *et al*. (2005) demonstrated resistance to some antimicrobial agents.

Meningococcal meningitis is an airborne and contagious disease caused by *Neisseria meningitis,* reported to occur globally. In the years 1996 to 1997, a meningitis belt stretched across sub-Saharan Africa, causing a major epidemic in history documenting over 250 000 cases, with approximately 25 000 deaths, and disability in 50 000 people (WHO, 2011). Although South Africa does not fall in the meningitis belt, high incidences have been reported from 1999 to 2002 in the Western Cape and Gauteng provinces as a result of serogroup B and A (Von Gottberg, 2008). The isolation of *Neisseria meningitis* on meat processing equipment was a clear indication of inadequate cleaning and disinfection of surfaces. Moreover, the organism can be transmitted by direct exposure to droplets and discharges from the nose and the throat of infected food handlers' contaminating the meat processing environments.

***Corynebacterium* spp.**

The genus *Corynebacterium* is widely distributed in nature and these bacteria are also part of the normal microbiota of human skin and mucous membranes (Gomila, 2012). The *Corynebacterium* branch of *Actinomycetales* encompasses 88 species that are known to colonize various environmental surfaces (Burkovski, 2013). *Corynebacterium cullunae* isolated from butchery E (Table 4.2) is a soil bacterium also known as a glutamic-acid-producing species (Fudou *et al.,* 2002.). The identification of opportunistic *Corynebacterium* pathogens relies on phenotypic methods such as molecular and/or biochemical techniques' (Hauser *et al.,* 1993; Khamis *et al.,* 2004). However, identifying species by molecular techniques is time consuming. Moreover in this study MALDI TOF-MS was used to distinguish potential toxigenic *Corynebacterium* spp. from harmless and/or opportunistic pathogens in order to make quick clinical decisions.

4.4.2.3 Yeast and fungi

Yeasts have a long history of safe usage in the food industry for the fermentation activities of bread and other food products. However, in meat and meat products they can cause spoilage as a result of hygiene negligence, or new processing and storage techniques (Fung and Liang 1990; Fleet, 1992). Literature on microbial spoilage of meat products attributes spoilage to bacteria rather than to yeast because of their more rapid growth rate on meat substrates at refrigeration temperature (Nortjé *et al*., 1990; Dillon, 1998; Kurtzman, 2006; Ercolini *et al*., 2006). Moreover, yeasts were previously considered insignificant due to their slower growth rate in chilled stored foods, making them less likely to compete with psychrophilic bacteria (Nortjé *et al*., 1990; Dillon, 1998).

The significant yeasts associated with meat products and isolated in this study, belong to the *Ascomycetous* and *Basidiomycetous* genera, *Candida* as well as *Rhodotorula*. Their occurrence is reported to be associated with fresh meat (Fleet, 1992; Osei *et al*., 2000).

MALDI-TOF MS has been recognized as a reliable, time-saving and cost-effective method compared to several molecular-based methods for identification of yeast. Regrettably, in this study the instrument emerged as a rapid and powerful tool for identification of pathogenic bacteria rather than for the identification of yeasts. Similarly, Pinto *et al.* (2011) agree that identification of yeast using MALDI-TOF MS is limited by the requirement for protein extraction and for robust reference spectra across yeast species in databases. Consequently, the shortcomings of MALDI-TOF MS in the fingerprinting of yeast species postulated a need for a more improved reference spectrum and database for yeast species which in recent times have been reported to cause infections ranging from blood stream to nosocomial, and also to cause a high mortality rate particularly in immune-compromised patients.

The wide distribution of the genus *Candida* contributes the most in number of species of the yeast genera; they are also part of the normal fungal flora of humans and animals. *Candida* is reported as an emerging fungal yeast pathogen causing acute, sub-acute or chronic yeast infections such as candidiasis in immune-compromised persons, and it

varies significantly from other yeasts due to its capability to cause disease. *Candida* species is also known to occur predominantly during the spoilage of meat.

Rhodotorula is a basidiomycetous species occurring widely in the air, soil, water, fresh and processed meat, fruits and vegetables and dairy products (Wirth and Goldani, 2012). Their rapid growth at refrigeration temperature is a potential source of spoilage in different foods stored at low temperatures. *Rhodotorula mucilaginosa,* isolated in butcheries D and E, has been shown to be the causative agent of skin infections in chickens and lung infection in sheep; it has also been found to cause skin lesions in a southern sea lion (Beemer *et al*., 1970; Alvarez-Perez *et al*., 2010).

Aureobasidium pullulans was also identified from the isolates (Table 4.2). This yeast-like fungus is ubiquitous in nature and can therefore be found in different environments, particularly where there are fluctuating water activities such as in bathrooms, soil, water, air, limestone and food. In this study, *A. pullulans* was isolated from the sink and floors which, according to observation, often appeared wet during sampling (Table 4.2). Since the genus *Aureobasidium* may also colonize hair, skin, and nails in humans, *A. pullulans* may be recognized as a contaminant *(*Taylor *et al*., 2006). Furthermore, A. *pollutants* has also been reported to be the causative agent of the cutaneous infection hypersensitive pneumonitis (Kurup *et al*., 1984; Pikazis *et al*., 2009; Joshi *et al*., 2010).

Among the fungi identified by MALDI-TOF MS in this study, *Trichophyton rubrum* is a fungus commonly reported to cause athlete's foot, jock itch, and ringworm (Cribier *et al.,* 1998). *Trichophyton rubrum,* which is known to be a human pathogen fungus, was isolated from the band saw surfaces in butchery A (Table 4.2). Studies have reported that *Trichophyton rubrum* is transmitted from person to person by shedding of infected skin cells and hair, as well as by direct body contact; hence, its isolation at a meat processing facility was a huge concern (Ohst *et al.,* 2004; Martinez *et al*., 2012).

4.5. Conclusion

High microbial loads on environmental surface samples suggest that the safety and quality of meat sold at the butcheries studied was at risk of being compromised. Moreover, the inadequately cleaned and disinfected processing equipment will inevitably cause product contamination during processing, possibly posing a risk to the consumer's health. Thus, identification of proper sanitizers which are effective on both clean and heavily soiled (with fat and tissue) surfaces is crucial to the success of consequent cleaning programmes. Butcheries should enforce careful scrutiny (microbiological methods) when assessing the hygiene status of environmental surfaces to reduce foodborne illnesses related to food of animal origin, with the possible implementation of food management systems such as general manufacturing practices (GMP), general hygiene practices (GHP) and hazard analysis and critical control points (HACCP).

The indirect contact surfaces such as floors determine the acceptability of the hygiene status of the food plant which was the case in the present study where all the butcheries showed high microbial loads on floors. The presence of spilled blood, stagnant water and the absence of foot baths in the butcheries are some of the significant factors promoting microbial growth on the floors with the possibility of biofilm formation. Therefore, management and meat handlers should be obliged to use foot baths before entering the premises to avoid bacterial floor colonization. Furthermore, lack of careful scrutiny (microbiological methods) when assessing the hygiene status of food contact surfaces, compromises meat safety extensively.

4.6. References

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Chapter 5

Quantification of microbial contaminants on hands and aprons of meat handlers in butcheries

QUANTIFICATION OF MICROBIAL CONTAMINANTS ON HANDS AND APRONS OF MEAT HANDLERS IN BUTCHERIES

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5.1 Abstract

Globally, the majority of foodborne illness outbreaks are associated with poor personal hygiene of food handlers. Hands of food handlers can unwittingly carry microorganisms from one place to another, causing cross-contamination and foodborne diseases. In this study, samples were collected from the hands and aprons of meat handlers in selected butcheries in the Mangaung Metropolitan municipal area. Analysis of the samples showed that the average bacterial load on male and female meat handlers' hands ranged from 9.78×10^{0} cfu.cm^{-2} to 2.89×10^{1} cfu.cm^{-2} and 1.22×10^{1} cfu.cm^{-2} to 4.01×10^{1} cfu.cm^{-2}, respectively. Counts on aprons ranged between 2.88×10^{1} cfu.cm^{-2} and 7.68×10^{1} cfu.cm^{-2}. The major bacterial pathogens isolated were *Escherichia coli*, *Staphylococcus aureus, Klebsiella* spp., *Pseudomonas aeruginosa* and *Bacillus cereus,* among others. This study was performed in order to determine the level of bacterial contamination on the hands and aprons of meat handlers. Lack of hand-washing resources in the studied butchery premises may promote foodborne illness outbreaks.

Keywords: *personal hygiene, cross contamination, organoleptic, butcheries food safety*

5.2 Introduction

Meat can transfer pathogenic organisms originating from unhealthy or carries animals to the food handlers who eat or handle it (South Africa, Department of Agriculture, 2000). Transmission of pathogenic microorganisms to food takes place through poorly washed hands and dirty clothing. Several studies have indicated poor personal hygiene as a major contributory factor of foodborne illness outbreaks (Collins 2001; Cogan *et al*., 2002; Mudey *et al*., 2010). The skin of the human being can be colonized by microorganisms. Moreover, emerging and microbial pathogen can change sequentially to match the environmental conditions which can be significant to their proliferation. Even a healthy worker may be a carrier of microbial pathogens on the skin, hands, hair and respiratory tract. Moreover, the microbial flora of the skin consists of resident and transient microorganisms. Resident organisms such as coagulase negative staphylococci can potentially survive and multiply in the superficial skin layers, while the transient microbial flora of the skin consists of recent contaminants that survive only for a limited period of time. These microorganisms include *Staphylococcus aureus*, *Escherichia coli*, and enterococci which may be acquired by contact with the normal flora of human and animal skin or colonised meat environment.

Food handlers' hands and fingernails as well as aprons play a crucial role in microbial transfer amidst meat and meat products and equipment. It is evident that faecal-related pathogens are transferred to food during handling by lack of hand washing after visiting the toilet (Drankiewicz and Dundes, 2003). Additionally, meat can also be contaminated

due to unconscious body habits of the food handler such scratching the nose, head and licking fingers, as 45-50% of the population is estimated to carry *Staphylococcus aureus* and *Escherichia coli* on the hands, nose and hair. Previous studies conducted from 1975 to 1998 in the food processing industries found the transfer of pathogenic microorganisms to food by food handlers to be the most common cause of 89% of foodborne illness outbreaks (Guzewich and Ross, 1999; Taylor *et al.,* 2000; Barza, 2004).

The importance of appropriate hand-washing in the reduction and prevention of pathogens acquired on hands and faces was first established by Doctor Ignaz Semmelweis in 1846. His discovery is used in medical healthcare and food industries to this day. Hand-washing may seem a simple thing, yet it is frequently taken for granted and forgotten. According to Challenge (2005) and the Education Foundation (National restaurant association) (2008), proper hand-washing entails wetting the hands and running warm water over them, lathering with soap, scrubbing for 10-15 sec, rinsing and air-drying or drying with paper towel. According to cultural practices in developing countries in Africa, including South Africa, most people eat their meals with hands and fingers especially at barbecues (braais). However, before eating, hands are often washed with cold water and without soap, which is not sufficient to ensure proper hygiene (Hoque, 2003).

This study was performed in order to determine the level of bacterial contamination on the hands and aprons of meat handlers. In this study, the effectiveness of washing hands with running water and soap was examined. The occurrence of bacteria found on hands and aprons was investigated with the use of MALDI-TOF MS. The purpose of the study was to determine whether current infrastructure and bacteriological analysis of personal hygiene is sufficient to prevent the meat sold in the selected butcheries from being contaminated with bacteria that could cause foodborne diseases.

5.3 Materials and methods

5.3.1 Sampling protocol

Each food handler was asked to wash his/her hands properly under running water with soap and to dry their hands with a paper towel in order to properly disinfect them. Eight samples were obtained from each meat handler's hands and apron. At least duplicate samples were taken from the left and right hands of the palms, forefingers, thumbs and also from their aprons, using RODAC plates. A total of 189 samples were collected from randomly selected food handlers and additional 28 samples were collected from the aprons during normal chores. Samples were collected from the same meat handlers throughout the study. The collected samples were kept in a cooler box at a very low temperature of 4-5ºC, and transported to the laboratory at the Central University of Technology, Free State, where they were incubated at a temperature of 25ºC for 3 days (Vorster *et al*.,1994).

5.3.2 Sampling procedure

A cross-sectional study was conducted in selected butcheries of Mangaung during the wet season as it is the high pick for public to buy their meat for holidays. Prior to sampling, randomly-selected meat handlers and managers were given an explanation of the purpose of obtaining samples from hands and aprons. Hands were washed prior to sample collection according to different guidelines per butchery. Because there were more males than females at the selected butcheries, two males and one female were selected for sampling purposes. RODAC plates of 55 mm (Merck, SA) containing non-selective media were used by trained surveyors, 5 times on a weekly basis to sample the forefingers, thumbs and palm of the right and left hand of each food handler. Aprons were sampled with RODAC plates on the areas that are predominantly exposed (six samples were collected per apron). The RODAC plate technique was found to be the best method of choice for the purpose of this study as the surfaces to be examined were smooth, firm, non-porous and were not heavily contaminated (Jay, 1992).

5.3.3 MALDI-TOF MS fingerprinting

Bacterial identification and fingerprinting was done efficiently in real time using MALDI-TOF MS which is equipped with a Microflex^{TM} LT benchtop, autoflex^{TM} with BioTyper^{TM}, COMPASS^{TM} for FLEX series incl. flexAnalysis^{TM} (Bruker Daltronics, Germany), at the genus, species and strain levels from each isolate. After incubation a piece of the colony was selected from biological material in the plate using a sterile loop, and submerged into an Eppendorf tube with 300 μl of ultra-pure water (Merck, SA).

Approximately, 900 *µ*l absolute ethanol was added carefully, vortexed, and centrifuged at the maximum speed of 13,200 × g for 2 min at room temperature. The supernatant was decanted and the pellet air-dried at room temperature. For extraction the dried pellets were mixed thoroughly by vortexing with 50 *µ*l formic acid (70%) (Merck, SA), followed by the addition of 50 *µ*l pure acetonitrile (Merck, SA) and further mixed thoroughly. The mixture was centrifuged at a maximum speed of 13,200 x g for 2 min, and approximately 1 *µ*l of the supernatant was spotted onto a 96 steel target plate (Bruker Daltronics, Germany) and allowed to air dry at room temperature. Samples on each spot were overlaid with 1 *µ*l of the HCCA matrix organic solvent mixture (a saturated solution of α-cyano-4-hydroxy-cinnamic acid (Sigma, USA) in 50% acetonitrile-2.5% trifluoro acetic acid) (Bruker Daltronics, Germany) and air dried at room temperature. The analysis of all strains was performed with a Microflex LT mass spectrometer (Bruker Daltronics, Germany) using Flex Control software (version 3.0, Bruker Daltronics, Germany). The spectra were recorded in the linear positive mode (with the laser frequency of 20 Hz; ion source of 1 voltage, 20kV; ion source of 2 voltage, 18.6 kV; lens voltage, 7.5 kV; mass range, 2000 to 20 000 Da). For each spectrum, 240 shots in 40-shots from different positions of the BTS spot (manual mode) were collected and analyzed. Prior to analysis, the spectra were internally calibrated using *Escherichia coli* protein extract as a test standard (Bruker Daltronics, Germany).

Spectra were obtained using a Microflex LT mass spectrometer (Bruker Daltronics, Germany) equipped with an N_2 laser. Sample preparation for MALDI-TOF analyses was carried out according to the OS-extraction protocol of Bruker Daltronics. The raw

spectra were imported into a dedicated BioTyper software (version 3.0, Bruker Daltronics, Germany), processed by standard pattern matching with standard settings, and the results reported in a ranking table with colour codes. Outcomes of the pattern-matching process were expressed as proposed by MALDI-TOF BioTyper (MT) manufacturer with ID scores ranging from 0 to 3. Scores <1.70 were considered not to have generated a reliable ID; a score of 1.7 <ID <1.9 was considered ID to genus, and a score >1.9 was used for reliable species ID.

5.4 Results and discussion

5.4.1 Total Viable Counts detected on hands and aprons

Total Viable Counts, which are used as indicators for microbiological quality of food, were detected on the hands and aprons of 75 food handlers [50 males (66.7 %) and 25 females (33.3%)]. Regulations 918 of 1999 promulgated under the South African Health Act 1977 (Act 63 of 1977) stipulates that any working surface that comes into direct contact with food, shall contain no more than 100 viable microorganisms per square centimetre upon analysis. Microbial levels of food handlers at butcheries are depicted in Figure 5.1. The average bacterial counts on the right hands of male meat handlers ranged from 1.12×10^{0} cfu.cm^{-2} to 9.33×10^{0} cfu.cm^{-2} with the highest total counts of 3.42×10^{1} cfu.cm^{-2} observed at butchery D and the lowest total counts of 9.78×10^{0} cfu.cm^{-2} observed at butchery B. On the left hands of male meat handlers, average bacterial counts ranged between 1.42×10^{0} cfu.cm^{-2} and 8.67×10^{0} cfu.cm^{-2} whilst the lowest total microbial counts were observed at butchery B (1.03×10^{1} cfu.cm^{-2}). The

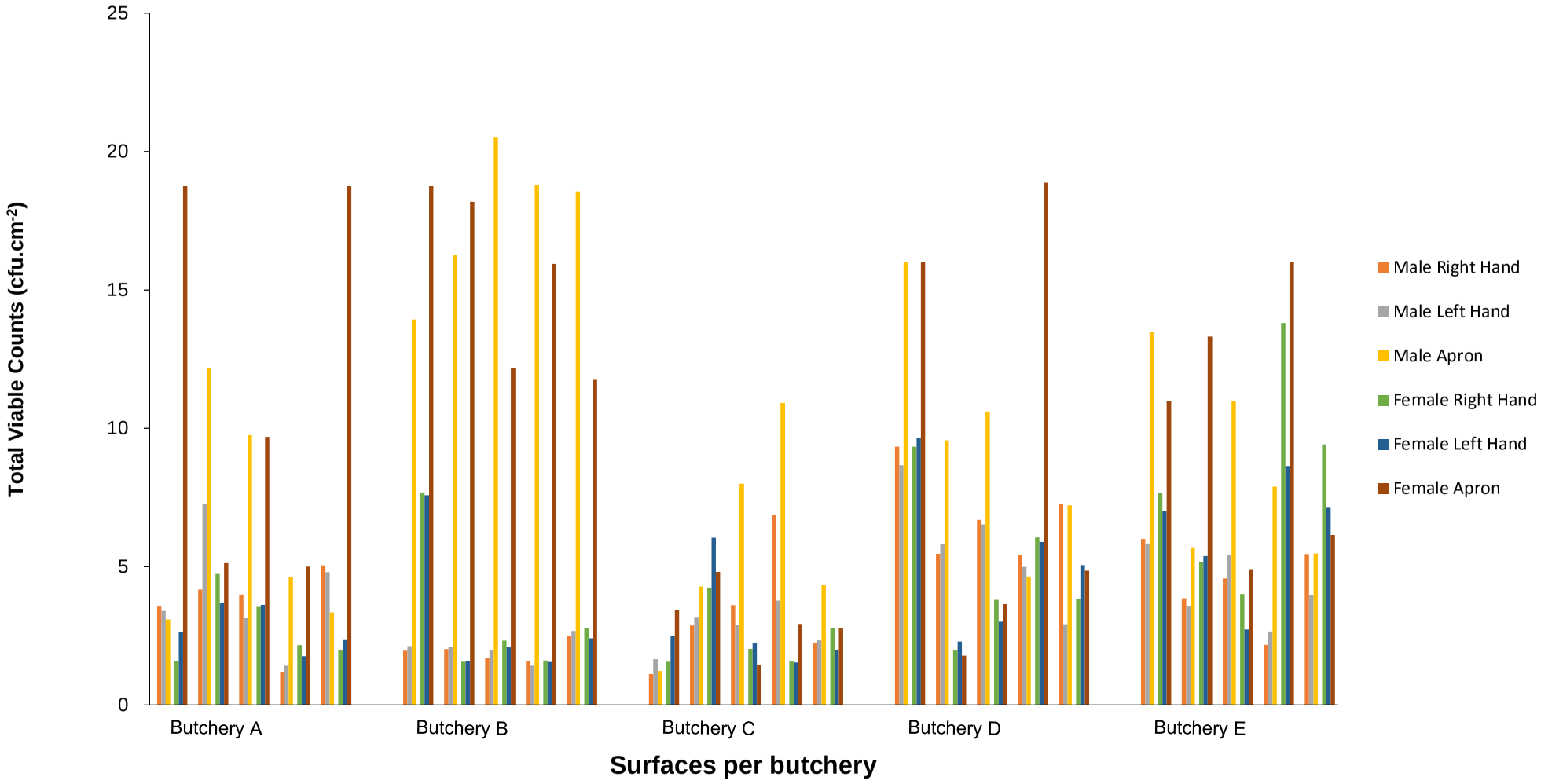

Figure 5.1: Total Viable Counts from aprons and hands of food handlers at selected butcheries over a five week period with intervals in between.

highest total counts of 4.8×10^1 cfu.cm^{-2} were observed at butchery D. A study conducted by Bloomfield and Scott (1997) found that transmission of 5.4×10^3 cfu.cm^{-2} to hands of food handlers during hamburger patty preparation was minimized by the use of antibacterial soap. In the current study, the results were lower than the results found in the study performed by Larson *et al.* (1998), in which total counts of bacteria on the hands of medical staff ranged from 3.9×10^4 cfu.cm^{-2} to 4.6×10^6 cfu.cm^{-2}.

Females showed the highest bacterial counts on the right hands ranging between 1.56×10^0 cfu.cm^{-2} and 13.8×10^0 cfu.cm^{-2}. The lowest total counts on females' right hands were observed at butchery C (1.22×10^1 cfu.cm^{-2}) whilst the highest total counts were observed at butchery E (4.01×10^1 cfu.cm^{-2}). The left hands of females had counts ranging between 1.54×10^0 cfu.cm^{-2} and 9.67×10^0 cfu.cm^{-2}. The lowest total counts were 1.41×10^1 cfu.cm^{-2} (butchery A) and the highest total counts were 3.09×10^1 cfu.cm^{-2}.

Aprons proved to have the highest counts throughout all the butcheries apart from butchery C where the lowest counts were observed on aprons throughout the study. Counts on aprons of both males and females ranged between 1.22×10^0 cfu.cm^{-2} and 20.5×10^0 cfu.cm^{-2}. Overall, male aprons appeared to be the filthiest with the lowest counts of 1.22×10^0 cfu.cm^{-2} (butchery C) and the highest counts of 20.5×10^1 cfu.cm^{-2} (butchery B). The lowest and highest total counts on male aprons were 2.88×10^1 cfu.cm^{-2} (butchery C) and 8.8×10^1 cfu.cm^{-2} (butchery B), respectively. Female

aprons showed the lowest counts of 1.44×10^0 cfu.cm^{-2} observed at butchery C and the highest counts of 19.0×10^1 cfu.cm^{-2} observed at butchery A. The lowest and highest total counts on female aprons were 1.54×10^1 cfu.cm^{-2} (butchery C) and 7.68×10^1 cfu.cm^{-2} (butchery B), respectively.

Lastly with regard to the total viable counts, the group data standard deviations STD_g = 3.0, 7.7, 2.7, 3.2 and 3.0 were recorded for all male meat handlers from butcheries A, B, C D and E respectively. Concomitant to this were the group data standard deviations for female meat handlers in butchery A, B, C, D and E recorded as STD_g= 5.7, 6.5, 1.3, 5.1 and 3.9 respectively. In addition, Table 5.1 below reflect significant differences between butcheries over the entire sampling period with regard to total viable counts quantified.

5.4.2 Microorganisms isolated

The bacterial contamination on hands and protective clothing of food handlers generally expose poor habits and practices of individuals' hygiene and the contamination of the final food product is a possibility (Olsen *et al.,* 2000; Montville *et al.,* 2002; Acikel *et al.,* 2008). Microorganisms collected on hands are characterized by the nature of handling (i.e. working activity). These microorganisms can attach to the outer garments worn in areas excluding the food or meat processing area such as restrooms or lunchroom, subsequently transmitting microbes to food contact surfaces (Barza 2004). The distribution of microorganisms on the hands and aprons of food handlers at the butcheries included Gram-negative bacteria, Gram-positive bacteria and yeasts (Table 5.2). The occurrence of *Enterobactericeae* family members (total coliforms and

Table: 5.1: Significant values for TVC counts within selected butcheries.

Butcheries grouping	**Significant value (p)**
A and B	0.017
A and C	0.0002
A and D	0.724
A and E	0.260
B and C	1.21×10^{-8}
B and D	0.0067
B and E	0.0006
C and D	0.00076
C and E	0.0082
D and E	0.435

Escherichia coli) and *Staphylococcus aureus* indicate a substantially increased risk of the presence of pathogens. Additionally, these microorganisms are widely used to measure or determine the effectiveness of sanitation programmes (Republic of South Africa: Department of Health, 2000).

Staphylococci

The genus *Staphylococcus* is composed of numerous species (40) of Gram-positive cocci. These bacteria are opportunistic pathogens carried on the hands, skin and in the nasal cavity of healthy individuals and animals (Epstein *et al*., 2009; Van der Haeghen *et al.,* 2011).

Staphylococcus aureus

In recent years, *Staphylococcus aureus* have emerged as a significant cause of several foodborne illnesses, often due to poor sanitation practices (Plata *et al.,* 2009; Schelin *et al.,* 2011; Rigby and De Leo 2012). Although this organism has been found as a commensal in approximately 30% of the skin and nostrils of humans (Lindsay and Holden, 2004; Gorwitz *et al.,* 2008), its presence in food is a major concern due to its resistance to antibiotics and its capability of producing toxins in various food products including meat, milk and eggs (Feng *et al*., 2008; Otto, 2010). In the current study *Staphylococcus* was detected from hands and aprons of meat handlers, which could possibly lead to post-processing contamination of meat and meat products. In order to control this species in meat environments it is possibly necessary to enforce the use of

disposable gloves as well as the use of proper hand washing methods. Moreover, the contamination of food contact surfaces with *Staphylococcus aureus* could be increased by ineffective cleaning and the formation of problematic biofilm formation in the food industry encouraging continual contamination of food (Götz, 2002).

Staphylococcus xylosus

Staphylococcus xylosus is a Gram-negative coccus and a commensal bacterium frequently found inhabiting the skin and mucous membranes of mammals and birds, as well as (occasionally) humans (Kloos, *et al.,* 1976; Nagase *et al*., 2002; Gozalo *et al*., 2010). Because *S. xylosus is* ubiquitous in nature, it has a greatly adaptive nature and is able to persist in soil and on surfaces possibly leading to the formation of biofilms in several environments (Kessie *et al*., 1998; Shale *et al*., 2005; Nimrat *et al.,* 2006; Planchon *et al.,* 2006). In the food industry, *S. xylosus* is used as a fermenting agent in the production of meat (sausage) and milk (cheese) products and it has been found to be a contributory factor in the organoleptic properties (taste, colour and aroma) of aforesaid food products (Montel *et al*., 1998; Talon *et al.,* 2002; Essid and Hassouna, 2013). Although the organism is recognized as non-pathogenic, some strains of *S. xylosus* may be opportunistic pathogens to animals and humans. Moreover, contamination of hands and aprons with this species could potentially be a food safety hazard with various implications.

Staphylococcus epidermidis

Staphylococcus epidermidis are coagulase-negative species and are among the most prevalent causes of nosocomial infections globally (Otto, 2009). In the present study, meat handlers' hands were found to be colonized by *Staphylococcus epidermidis,* which is recognized as an opportunistic pathogen in humans with bacteraemia, urinary-tract infections as well as post-catheterization (Martineau *et al.,* 1996; Burnie and Loudon, 1997). *Staphylococcus epidermidis* is part of the epithelial microflora and mucous membranes of warm blooded animals and humans, with probiotic properties hindering colonization of other pathogenic microbes (Duguid *et al.,* 1992*;* Nilsson *et al.,* 1998).

Staphylococcus intermedius

Staphylococcus intermedius, isolated at butchery B (Table 5.2), is coagulase-positive and is an opportunistic pathogen of pigeons, dogs, foxes, mink and horses (Hájek, 1976). Numerous infections such as otitis externa, pyoderma, abscesses, reproductive tract infections, mastitis, and canine-inflicted human wound infections may be ascribed by this zoonotic species (Goldstein, 1992; Lee, 1994). The isolation of this species in the current study raised concerns as they are commonly isolated from the skin, oral, or nasal flora of healthy dogs. Additionally, Talan *et al.* (1989) reported that *Staphylococcus intermedius* are rarely isolated from human beings including those who are in constant contact with dogs. In the year 1991, *Staphylococcus intermedius* was reported to be the etiologic agent in an outbreak associated with a butter spread,

Table 5.2: Microbial profile isolated from food handlers' hands and aprons at butcheries

Items	Strains isolated	Butcheries				
		A	B	C	D	E
Male Left Hand	*Acinetobacter* sp. DSM 14965DSM				X	
	Acinetobacter johnsonii DSM 6963T HAM	X				
	Acinetobacter radioresistens LMG 10614 HAM	X				
	Acinetobacter lwoffii B101UFL			X		
	Acinetobacter johnsonii DSM 6963T HAM				X	
	Bacillus cereus 994000168 LBK	X		X	X	
	Bacillus cereus DSM 31T DSM				X	
	Bacillus mojavensis DSM 9205T DSM			X		
	Bacillus pumilus DSM 1794 DSM					X
	Bacillus subtilis ssp. *Subtilis* DSM 5660 DSM			X		
	Clostridium difficle MB786905 THL			X		
	Enterococcus faecalis 202474 CHB		X			
	Escherichia coli ATCC 35218 CHB		X			
	Klebsiella bavingbed 37924 PFM					X
	Lactobacillus delbrueckii spp. *Lactis* DSM 20073 DSM		X			
	Lactobacillus delbrueckii spp. *Lactis* DSM 20355DSM		X			X
	Lactococcus garvieae DSM 20684T DSM		X	X		
	Lactobacillus lactis spp. *Lactis* DSM 20384 DSM					X
	Lactobacillus lactis spp. *Lactis* DSM 20175 DSM					X
	Lactobacillus reuteri DSM 20016T DSM					X
	Macrococcus caseolyticus DSM 20597T DSM		X			
	Pseudomonas aeruginosa ATCC 27853 THL				X	
	Pseudomonas fragi DSM 3456T HAM			X		
	Staphylococcus epidermidis ATCC 12228 CHB				X	
	Staphylococcus epidermidis ATCC 12228 THL				X	
	Staphylococcus epidermidis 10547 CHB				X	
	Staphylococcus epidermidis DSM 3269 DSM	X				
	Staphylococcus hominis spp. *Hominis* DSM 20328T DSM		X			
	Staphylococcus hominis spp. *Hominis* DSM 20330 DSM	X				
	Staphylococcus equorum ssp. *Equorum* DSM 20675 DSM			X		
	Staphylococcus pasteuri DSM 10657 DSM				X	
	Staphylococcus simiae DSM 17637 DSM		X			
	Staphylococcus vitulinus DSM 9930 DSM					X
	Staphylococcus warneri DSM 20036 DSM				X	
	Staphylococcus warneri Mb18796_1 CHB		X	X		
Male Right Hand	*Acinetobacter lwoffii* 2Ring 240 MHH		X	X		
	Acinetobacter johnsonii 31 PIM	X				
	Acinetobacter tjernbergiae DSM 14971T HAM	X				
	Acinetobacter radioresistens LMG 10614 HAM	X				
	Acinetobacter radioresistens DSM 6976T HAM		X			
	Staphylococcus aureus ATCC 25923 THL	X			X	X
	Staphylococcus pasteuri DSM 10657 DSM	X				
	Bacillus cereus DSM 31T DSM	X		X	X	

	Bacillus flexus DSM 1320T DSM			X		
	Bacillus subtilis DSM 5552 DSM			X		
	Escherichia coli DH5alpha BRL		X			
	Escherichia coli MB114641 CHB		X			
	Klebsiella cavingced 37585 PFM	X				
	Lactobacillus lactis spp. *Cremoris* DSM 20388 DSM					X
	Lactobacillus lactis spp. *Lactis* DSM 20661 DSM					X
	Lactobacillus murinus DSM 20453 DSM					X
	Lactobacillus reuteri DSM 20016T DSM			X		
	Pseudomonas aeruginosa ATCC 27853 THL		X			
	Serratia marcescens DSM 12483 DSM				X	
	Staphylococcus aureus spp. *Aureus* DSM 3463 DSM		X			
	Staphylococcus epidermidis ATCC 12228 CHB			X		
	Staphylococcus epidermidis DSM 1798 DSM		X		X	
	Staphylococcus hominis Mb 187881 CHB					X
	Staphylococcus hominis spp. *Hominis* DSM 20330 DSM	X				
	Staphylococcus intermedius DSM 20373T DSM		X			
	Staphylococcus warneri DSM 20036 DSM		X			
	Staphylococcus warneri Mb187961 CHB		X			
	Staphylococcus xylosus DSM 6179 DSM			X		
Male Aprons	*Acinetobacter ursingii* DSM 16037T HAM					X
	Aureobasidium pullulans 12235 CBS		X			X
	Aureobasidium pollulans 15131 CBS	X				
	Bacillus atrophaeus DSM 675 DSM			X		
	Bacillus cereus DSM 31T DSM	X				
	Bacillus megaterium DSM 32T DSM				X	
	Bacillus subtilis ssp. *Subtilis* DSM 10T DSM			X		
	Bacillus thuringiensis DSM 2046T DSM			X		
	Enterococcus faecalis ATCC 29212 CHB	X				
	Escherichia coli ATCC 25922 THL		X			
	Klebsiella cavingced 37924 PFM		X			
	Klebsiella cavingced spp. *cavingced* 92951 CHB	X				
	Pseudomonas aeruginosa ATCC 27853 THL	X				
	Pseudomonas fragi DSM 3456T HAM		X			X
	Pseudomonas lundensis DSM 6252T HAM					X
	Staphylococcus aureus ATCC 25923 THL					X
	Staphylococcus hominis spp. Novobiosepticus DSM 15614T DSM		X			
	Streptomyces lavendulae B264 UFL			X		
Female Left Hand	*Acinetobacter radioresistens* DSM 6976T HAM		X			
	Bacillus cereus 994000168 LBK				X	
	Bacillus cereus DSM 31T DSM	X				
	Bacillus megaterium DSM 32T DSM	X			X	
	Candida kefyr[ana] (Kluyveromyces marxianus spp. *marxianus)* CBS 834 CBS		X			
	Lactobacillus delbrueckii spp. *Bulgaricus* DSM 20081T DSM		X			
	Lactobacillus reuteri DSM 20053 DSM			X		
	Lactococcus lactis spp. *Cremoris* DSM 20388 DSM					X
	Lactococcus lactis spp. *Lactis* DSM 20661 DSM					X
	Macrococcus caseolyticus DSM 20597T DSM		X			

	Micrococcus luteus 59 PIM				X	
	Micrococcus luteus BK0114009 ERL					X
	Pseudomonas aeruginosa ATCC 27853 THL		X			
	Pseudomonas aeruginosa DSM 50071T HAM	X				
	Pseudomonas taetrolens LMG 2336T HAM			X		
	Serratia marcescens 131031 CHB				X	
	Staphylococcus aureus ATCC 33862 THL		X			
	Staphylococcus aureus spp. *Aureus* DSM 4910 DSM	X				
	Staphylococcus caprae DSM 20608T DSM	X				
	Staphylococcus epidermidis DSM 1798 DSM		X			
Female Right Hand	*Bacillus megaterium* DSM 32T DSM				X	
	Bacillus thuringiensis DSM 2046T DSM	X				
	Candida parapsilosis ATCC 27853 THL		X			
	Citrobacter freundii 13158_2 CHB			X		
	Lactobacillus reuteri DSM 6333 DSM					X
	Lactobacillus sakei DSM 6333 DSM					X
	Lactococcus garvieae DSM 20684T DSM					X
	Lactococcus lactis spp. *Lactis* DSM 20384 DSM		X			
	Lactococcus lactis spp. *Lactis* DSM 20661 DSM		X			X
	Moraxella sg Moraxella canis DSM 18277T DSM				X	
	Neisseria weaven DSM 17688T DSM		X			
	Pseudomonas aeruginosa ATCC 27853 THL	X				
	Pseudomonas aeruginosa DSM500071T HAM			X		
	Pseudomonas fragi DSM 3456T HAM			X		
	Pseudomonas lundensis DSM 6252T HAM			X		
	Raoultella ornithinolytica MB18887 CHB			X		
	Serratia marcescens DSM 12483 DSM				X	
	Serratia marcescens ssp. *Marcescens* DSM 30121T DSM				X	
	Staphylococcus aureus ATCC 33862 THL		X			
	Staphylococcus epidermidis DSM 3269 DSM		X			
	Staphylococcus epidermidis DSM 1798 DSM	X				
	Staphylococcus haemolyticus 19 ESL		X			
Female Apron	*Acinetobacter lwoffii* 54 PIM					X
	Bacillus thuringiesis DSM 2046T DSM				x	
	Enterobacter cloacae 201052 CHB			X		
	Lactobacillus coryniformis ssp. *Torquens* DSM 20005 DSM			X		
	Lactococcus lactis spp. *Lactis* DSM 20384 DSM					X
	Micrococcus luteus 59 PIM					X
	Pseudomonas aeruginosa DSM 50071T HAM		X			
	Pseudomonas lundensis DSM 6252T HAM		X			
	Staphylococcus aureus ATCC 25923 THL				X	
	Staphylococcus capitis ssp. *Capitis* DSM 20325 DSM	X				
	Staphylococcus hominis Mb187881 CHB		X			

affecting 265 people in the United States of America (Khambaty *et al.,* 1994; Vandenesch *et al.,* 1995),

Escherichia coli

Among Gram-negative bacteria, *Escherichia coli* are intestinal organisms largely reported to be harboured on human hands (South Africa, Department of Health, 2000; Shojaei *et al.,* 2005). Isolation of *E. coli* in this study from hands and aprons is a clear indication of poor personal hygiene practices such as not washing hands properly after visiting the lavatory. Although De Wit and Rombust (1992) report that *E. coli* in general is seldom present on hands, the current study was in agreement with the study performed by Lues and Van Tonder (2006) who found 40% occurrence of *E. coli* on the hands. Moreover, the hands of meat handlers from this establishment (butchery B) may have been contaminated with this organism during meat processing, resulting in the hands being contaminated with a high density of enteric pathogen (Taylor *et al.,* 2002).

Pseudomonades

Pseudomonades are food-spoilage, transient microorganisms deposited through direct contact with the contaminated surface or aerosolization. Pseudomonades are Gram-negative bacteria occurring commonly in water and soil. These organisms can proliferate in very low temperatures which may be a problem in the meat industry where low temperatures are a necessity.

Pseudomonas aeruginosa

Pseudomonas aeruginosa was predominantly isolated on females' hands and aprons which was a concern (Table 5.2). It is known to be a causative factor of urinary tract, blood stream and chronic lung infections in cystic-fibrosis patients (Muyldermans *et al.,* 1998; Zawacki *et al*., 2004). In the present study, *P. aeruginosa* it was observed on many occasions that some females were wearing nail polish and artificial fingernails, resulting in less effective hand washing, as well as facilitating the colonization of microorganisms on the hands. Moreover, several studies indicate that artificial fingernails amongst healthcare workers (HCWs) are a risk factor for colonization of hands with *P. aeruginosa* (Foca *et al.*, 2000; Moolenaar *et al.,* 2000). Requiring short natural fingernails in meat processing establishments is a reasonable policy that may be implemented to reduce the incidence of infections associated with *Pseudomonas* species at butcheries.

Pseudomonas aeruginosa has been reported to proliferate in dispensers of liquid hand soap in elementary schools, causing infections leading to mortality (Zapka *et al.,* 2011). In recent times, it has been demonstrated that using an acceptable hand-washing method, as suggested by the World Health Organization (WHO), may reduce the risk associated with the contamination of hands with *P. aeruginosa* even though the water utilized during hand washing may possibly be profoundly contaminated with this organism (Pittet *et al.,* 2009; Jones, 2011).

Klebsiella

Klebsiella species are Gram-negative bacteria that are known to cause health care-associated infections. These bacteria are ubiquitously found in the environment (animal and human faeces, soil, surface water, sewage, grains, fruits and vegetables) (Podschun *et al*., 2001). In recent years, *K. pneumoniae* has become a major health problem worldwide due to the emergence of strains resistant to carbapenem antibiotics (Yigit *et al.,* 2001). *Klebsiella pneumoniae* is part of the normal flora of the gastrointestinal tract (Lau e*t al*., 2008). However, in large numbers it can be an opportunistic pathogen and a causative agent of bacteraemia, pneumonia, urinary tract infections and other human infections (Hussein *et al.,* 2013). Transmission of *K. pneumoniae* in the hospital environment is through blood products, contaminated medical equipment, the gastrointestinal and respiratory tracts of patients, and the hands of hospital personnel (Podschun and Ullmann, 1998).

Acinetobacter

The *Acinetobacter* species are Gram-negative, coccobacillary bacteria (Bergogne-Berezin and Tower, 1996). *Acinetobacter* species are resident microorganisms colonizing up to 43% of healthy adults' skin and mucous membranes, particularly in moist regions such as the axillae, groin, and toe webs (Seifert *et al.,* 1997). Because they are ubiquitous in nature and can attach to different environments surviving adverse conditions, they have emerged as major cause of nosocomial infections and other mild to severe illnesses, sometimes leading to fatality (Joly-Guillou, 2005). In this study,

the hands and the aprons of food handlers were colonized with *Acinetobacter ursingii* DSM 16037T HAM, *Acinetobacter lwoffii* 54 PIM, *Acinetobacter* sp. DSM 14965 DSM, *Acinetobacter johnsonii* DSM 6963T HAM, and *Acinetobacter tjernbergiae* DSM 14971T HAM. *Acinetobacter* is reported to be a common bacterial species causing spoilage in chilled meat among others (Pin and Baranyi, 1998). In addition, food products such as vegetables, apples, melons, mushrooms, radishes, and cereals such as sweetcorn are also known to be prone to spoilage by *Acinetobacter* species (Berlau *et al.,* 1999, Peleg *et al.,* 2008).

***Bacillus* spp.**

Among the *Bacillus* species isolated on the hands and the aprons of food handlers, *Bacillus cereus* was the organism predominately isolated in the present study. *Bacillus cereus* is a Gram-positive spore former, widely distributed environmentally in the soil and air, and favourable to the adverse conditions of the food production environment, therefore possibly contaminating a variety of food products (Jensen *et al.,* 2003; Stenfors *et al.,* 2008). *Bacillus cereus* has also been found to have the ability to grow well in the intestinal tract of insects and mammals and can also colonize the human intestine (Stenfors *et al*., 2008).

The route of contamination of meat and meat products with this organism (other than by transmission via food handlers' hands) could be due to the presence of spores in the air and their resistance to heat (Ribeiro *et al.,* 2010). Cases of foodborne illness caused by

Bacillus cereus are under reported because of their mild symptoms. The variety of foods contaminated with *Bacillus cereus* includes meats, milk, vegetables and fish, which have been associated with diarrhoeal-type food poisoning (Agata *et al.,* 2002). Rice products, cheese and pasta have also been associated with vomiting-type outbreaks, through eating food prepared by infected hands, thus posing health risks (Jay, 1998).

Neisseria spp.

All members of *Neisseria* spp. Have traditionally been described as Gram-negative cocci and non-spore-forming bacteria that are commensal flora of the mucous membranes of the oropharynx and upper respiratory tract in humans and animals (Janda and Gaydos, 2007; Virji, 2009), rarely causing diseases. However, the two most recognized members of the *Neisseria* genus are *Neisseria meningitides* and *Neisseria gonorrhoeae,* which are reported to be pathogenic (Stabler *et al.,* 2005). These are reported as important human pathogens known to cause bacterial meningitis, gonorrhoea and urethritis (Wong and Janda, 1992; Michaux-Charachon *et al*., 2005). In the current study, *Neisseria weaveri* was isolated in butchery B, was previously named CDC group M-5 (Andersen *et al*., 1993). *Neisseria weaveri* is part of the normal flora of the upper respiratory tract in dogs (Carlson *et al*., 1997). The isolation and infectious complications associated with *N. weaveri* in humans such as wound bacteraemia, pneumonia, peritoneal dialysis and catheter-related infections, are as a result of dog and cat bites (Carlson *et al*., 1997; Panagea *et al*., 2002; Kocyigit *et al*., 2010). In this study the isolation of *N. weaveri* indicates a lack of clinical evaluations of food handlers

in the food processing establishment. It further suggests poor illness reporting and a weak communication system between the workers and their managers.

5.5 Conclusion

Despite the fact that all the meat handlers were requested to wash their hands prior to sampling, the levels of bacterial loads showed negligence on the part of meat handlers in the studied butcheries. The faecal-oral route has been highlighted as the most frequent route of worker contamination (Todd *et al.,* 2009). In the present study, findings indicate limitations at hand-washing stations where it was observed that no water (neither hot nor cold) was conveniently available to the workers, possibly increasing the transmission of microbial counts to hands as well as to protective clothing. Because protective clothing of food handlers can transmit microbes from different sources of food products, the Meat Safety Act of 2000 (Act no. 40 of 2000) (Republic of South Africa, 2000) clearly stipulates that clean protective clothing should be provided to employees so as to maintain good hygiene status.

The results of the current study (as reported in Chapters 2 and 3) show that employee health and hygienic practices might have a direct impact on the safety of foods and the spread of microorganisms from worker to worker as well as to the final product. High counts were observed from aprons and hands of food handlers, suggesting that hand-washing sinks should be conveniently located for food handlers (butcheries B and E). The high counts of microorganisms on the aprons of both males and females in

butcheries A, B, D, and E could be attributed to the fact that the meat handlers launder their own protective clothing after work in their homes without proper monitoring of the laundering. Unlike abattoirs, butcheries make no provision for laundry facilities for the washing and drying of linens, cloths, uniforms and aprons necessary to the operation of the food establishment.

The study further revealed that there is a reasonable gap in practices regarding proper hand hygiene among meat handlers in butcheries. Additionally, the impact of bacteria on meat resulting in putrefaction, sourness and rancidity, including food or meat poisoning, suggests a need for the training of cleaning staff and the execution of rapid hygiene assessment. Self-inspection in the studied butcheries will be great tool for managers and the food handlers to ensure that their facility follows personal and general good hygiene practices. Moreover, it is important to note that microorganisms can be transmitted from meat to the people who eat and handle meat.

5.6 References

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Chapter 6

Rapid food contact surface hygiene analysis using ATP bioluminescence in butcheries

RAPID FOOD CONTACT SURFACE HYGIENE ANALYSIS USING ATP BIOLUMINESCENCE IN BUTCHERIES

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6.1 Abstract

Inspection of the butchery premises prior to meat processing is an important step in the production of quality and wholesome meat for human consumption. Adenosine triphosphate (ATP) bioluminescence is utilized to rapidly monitor the surface hygiene, giving results in seconds. Limitations of the existing methods to assess environmental surface (visual inspection), and the regulations that govern the method, make objective evaluations difficult in terms of microbial surface colonization, possibly affecting the safety and quality of meat. Therefore the aim of this study was to examine the residual contamination associated with direct and indirect meat contact surfaces to be able to determine in a short space of time the acceptability of hygiene levels in selected butcheries. In this study, surface cleanliness at butcheries was monitored using ATP bioluminescence expressed in relative light units (RLUs) to provide instant results of the hygiene status of 18 meat contact surfaces assessed in duplicate against the benchmark of 100 relative light units. Although the level of RLUs differed between items and butcheries, the surfaces of hooks, sinks, band saws, floors and scales revealed the highest dirt levels, exceeding 1000 RLUs. The results highlighted the fact that the butchery surfaces were of an inadequate hygiene status, and suggested a need for continuous training of staff members in cleanliness of food contact surfaces. The effectiveness of cleaning and sanitation in this study cannot be overemphasized particularly in view of the regulations governing the implementation of the hazard analysis critical control point (HACCP) system.

Keywords: *ATP bioluminescence, equipment, cleaning and sanitation, monitoring*

6.2 Introduction

The persistence of some foodborne pathogens on food contact surfaces affects the quality and safety of the food products. Numerous reports have indicated that contamination of meat is caused by improper cleaning and disinfection of equipment (Gibson *et al.*, 1999; Jessen and Lammert, 2003; Ali *et al.,* 2010). The effect of disinfection of food contact surfaces strongly depends on the amounts of protein and fat residues present on the utensils and equipment. The high content of protein and moisture potentially make meat an ideal medium for the development and reproduction of microbes such as bacteria that can cause spoilage (Jackson and Megowan, 2001; Mayr *et al.,* 2003; Dave and Ghaly, 2011). Furthermore, rapid growth of various organisms can be expected on the meat environmental surfaces unless effective control and monitoring is achieved. Inadequate cleaning may lead to the formation of biofilms, causing a challenge to the production of wholesome products (Maukonen *et al*., 2003; Beauchamp *et al.,* 2012).

Although effective cleaning of food contact surfaces is considered one of the most important activities in the meat plant, little has been done to provide results that will provide immediate corrective action particularly in butchery environments (Carling and Bartley, 2010). Therefore, the assessment of cleaned surfaces prior to and after food processing has been identified as an integral part of the quality control programme in order to reduce the possible risks to public health due to food contamination. Food contact surfaces, and non-contact surfaces such as floors, must be cleaned to remove food residues, soils, dirt and residential flora that could provide nutrient sources for

microbial growth (Dancer, 1999; Griffith *et al*., 2000). The degree of microbial contamination that occurs on food contact surfaces depends largely on the hygiene status of the employees and their food handling behaviour as well as their practices, (Gill and Jones, 1999). Hence, adenosine triphosphate (ATP) is a good, rapid, environmental monitoring technique to measure organic soils or debris persisting on food contact surfaces due to ineffective cleaning (Costa *et al*., 2006).

Although microbiological guidelines exist to minimize microbial safety hazards associated with food contact surfaces, results from the traditional microbial plate method require lengthy periods to obtain results, thus offering little help as part of hygiene monitoring. Rapid detection methods such as ATP were developed with the objective of obtaining results in real time and have been utilized successfully for years. Moreover, the ATP method is widely recognized as a rapid cleanliness monitoring analysis and its measurement provides an indication of the both microbial and non-microbial soils on surfaces. Additionally, the ATP measure the effectiveness of the cleaning procedures in place (Kyriakides and Patel, 1995; Illsey *et al*., 2000; Bellamy, 2012). This method detects the amount of ATP on the food contact surface by evaluating the hygiene status. Adenosine triphosphate reacts with the enzyme on the swab luceferin-luciferace and emits light as a relative light unit (RLU). However, ATP cannot be a replacement for traditional microbiology as it only detects food residues and not bacteria (Griffith *et al*., 2005). The device allows managers to verify the hygiene status of their food processing plants as a stand-alone method, independent of the presence of inspectors or a microbiologist in real time, thus being ideal for the food

processing industry. Therefore the device is of special importance to premises such as butcheries that employ the services of EHP (Environmental Health Practitioners) who usually assess the cleanliness through visual inspection. According to Moore and Griffith (2002), visual inspection can only reveal contamination risks and gross soiling, but fails to reveal contamination by microorganisms which can only appear with an aid of microscope and/or other microbiological methods.

Detergents and disinfectants used for food contact surfaces should be used at manufacturer-recommended concentrations and instructions to ensure their effectiveness. Numerous studies conducted in food premises have indicated that uncontrolled disinfectants may affect food quality and safety as a result of chemical residue contaminants, increasing incidences of contamination associated with meat (Tebbutt, 1991; Green *et al*., 1999; Bremer *et al*., 2006). The aim of this study was, therefore, to evaluate the cleanliness of food contact surfaces rapidly by using ATP bioluminescence and to help establish a cleaning protocol for food contact surfaces.

6.3 Materials and methods

6.3.1 Sampling site

Samples were collected from five processing areas of selected butcheries within the Mangaung Metropolitan municipal area in the Free State province, of South Africa. The butcheries were selected based on recommendations from Environmental Health

Practitioners of Mangaung Metropolitan Municipality. Fifteen percent (15%) of the butcheries were selected as they are the most preferred by consumers in the jurisdictional area and three of them also faced some challenges as reported by the officials. It must also be noted that not all butcheries had all equipment's but all sampled items per butchery are reported as more than 90% were present in most butcheries.

6.3.2 Sampling protocols

At least duplicate samples were collected from environmental surfaces in the mornings, prior to commencement of usual daily duties and all surfaces were subjected to basic cleaning procedures which included the use of detergent and sanitation with high pressure water. Samples were collected between the months of October and December with two week intervals between sampling Specialized (ultra-snap) swabs (Figure 6.1) were used to sample a standardized area of 5×5 cm^2 per surface area. The swabs were pre-moistened with enzyme compound luciferase and the reaction was shaken and activated by the enzyme luciferase that uses chemical energy contained in the ATP molecule to drive the oxidative decarboxylation of luciferin, with the resultant production of light.

6.3.3 Sampling procedure

In order to assess the level of surface cleanliness, this study used a quantitative microbiology data sampling method (Kaivac Cleaning Systems, 2011). At start-up, the

Figure 6.1: ATP bioluminescence portable machine and swabs (Hygiena, 2014)

hygiene luminoter was allowed to self-calibrate at a constant temperature and same location to reduce errors. After swabbing selected surface areas, samples were transported to the laboratory at low temperatures of between 0ºC and 4ºC. The bulb of the swab was snapped twice to three times and liquid-stable reagent squeezed down the tube to bathe the swab bud by a gentle shaking motion. The activated ultra-snap was inserted into the device. Light was emitted in direct proportion to the amount of ATP present and measured in RLU. The higher the RLU reading, the greater the level of ATP present, representing a tangible measurement of organic soil on the environmental samples (Malik *et al.,* 2003). According to the Hygiena®ATP manufacturer's recommendations (2014), the following readings in relative light units were considered: ultra clean r from 0-10, very clean 11-30, good clean 31-80, somewhat dirty 81-200 (Kaivac Cleaning Systems, 2011).

6.4 Results and discussion

In total, 75 surfaces were sampled across all the five butcheries. Sites were selected to include those with high frequency of contact by staff and with the potential to be involved in cross-contamination routes. The presence of adenosine triphosphate (ATP), which is derived from microorganisms and organic soil, was assessed at each site by a rapid hygiene test of ATP bioluminescence (Figure 6.1), using the Hygiena® ATP system sure II (Microsep (Pty) Ltd, South Africa). ATP levels for all the sampled areas or items varied for all five of the selected butcheries.

Generally the level of contamination was higher on the frequently used equipment surfaces such as hooks, sinks, sealers, band saw machines and the floors from all the butcheries. The maximum and minimum values on the surface samples that were measured by ATP bioluminescence are presented in Figure 6.2. The results indicate that the hygiene quality of the meat processing surfaces were not of a satisfactory standard. According to the manufacturer's recommendation, the organic soil with ATP levels of >100 designate denotes 'hygiene failure' and ATP levels of >1000 are considered the filthiest. Ten items in butchery A (which included: band saw blade and surface, hooks, scoops, sink, sealer, knives, trays, floors, containers) emitted more than 1000 RLUs on average, indicating filthiness, while the scales from the processing area, mincer and surfaces of the meat slicer showed levels lower than 100 RLUs on average.

The group standard deviation values in butchery A was STD_g=1240.47 with the highest RLUs were obtained on the band saw blade and hooks after cleaning, which respectively recorded the values of 1849.6 RLUs and 1517.0 RLUs on average. This could be attributed to organic materials that are not eradicated completely after cleaning with disinfectants, leading to high bacterial contamination on these surfaces. This was of great concern since the surfaces were made of stainless steel which is known to be easy to clean and disinfect (Krysinski *et al*., 1992). In a study conducted by Wildbrett and Sauerer (1989), proteins attached to stainless steel were found to be the main fouling component. Despite the importance of sanitation within the food processing environment, foodborne illness outbreaks associated with products produced under unsanitary conditions continue to be reported. For instance, in the year 2008, an

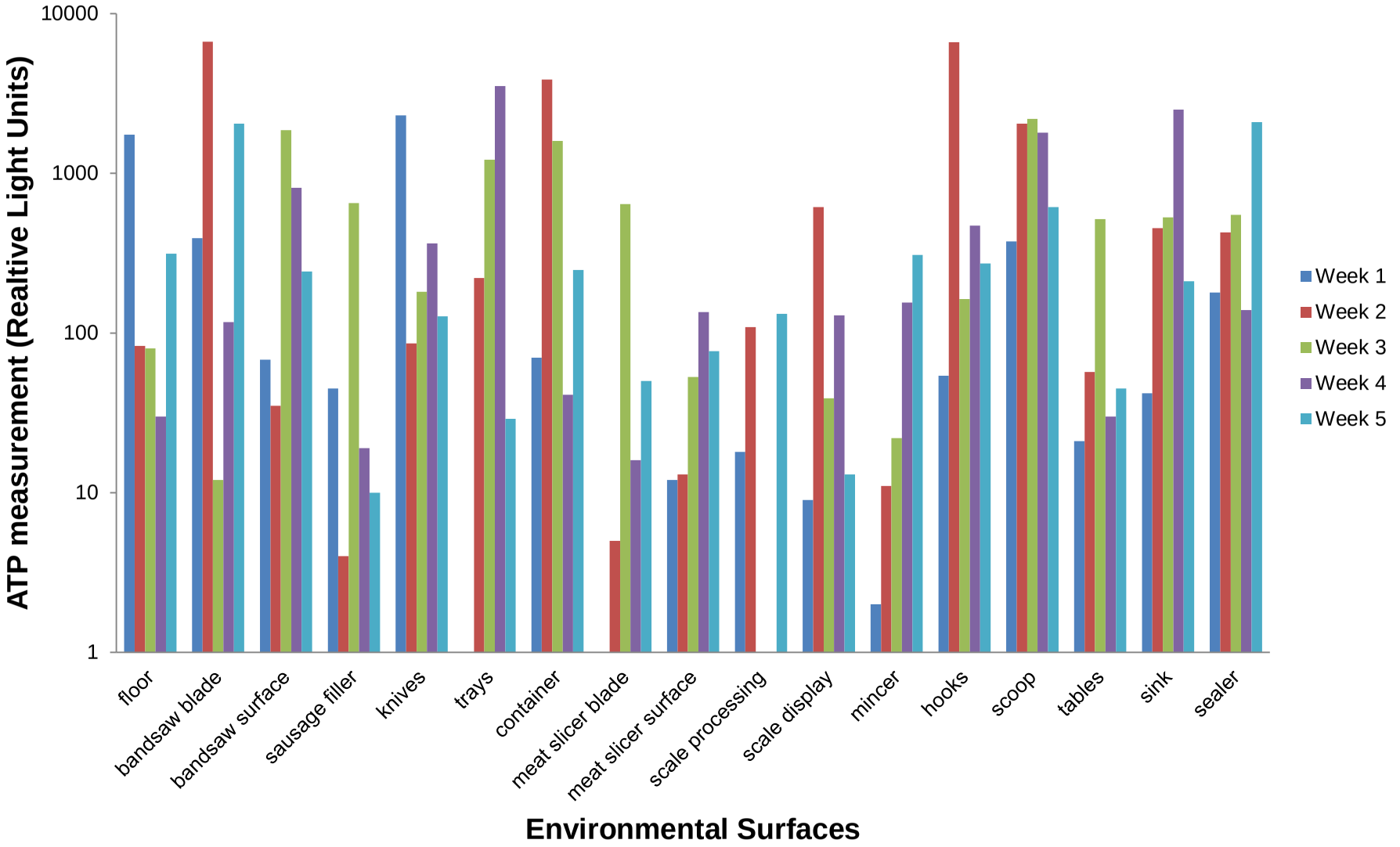

Figure 6.2 : ATP assay on environmental surfaces at butchery A

outbreak of listeriosis was found to be related to deli meat contaminated as a result of an insufficiently cleaned and sanitized meat slicer (Benjamin *et al.*, 2012). An important finding in a study conducted by White *et al.* (2008) was that poor cleaning and sanitation practices related to equipment was found to be attributable to work pressure in the food establishments. This was in agreement with the visual observation as the establishment was considered the cheapest and most preferred, as shown by its busy schedule throughout the day.

Floors showed slightly higher levels of 451.2 RLUs on average, which levels were possibly enhanced by the use of the sawdust to absorb any liquid spills. However, the clean-up does not get easier after all the blood spills from various meat pieces/parts. During the administration of questionnaires (Chapter 2), the meat handlers mentioned that though the sawdust prevented them from slipping due to the spills on the floor, it was not swept up and/or replaced each day; the build-up of microorganisms through unsanitary floors was therefore increasing. According to NRS 446.841 (2011), the use of sawdust is applicable in food facilities such as butcheries where activities such meat cutting, deboning and packaging are engaged though it must be replaced every day.

The low level of 52.2 RLUs on the processing scales could be due to the fact that meat is not directly weighed on the scale platform, but rather weighed in a plastic bag. Additionally, the scale platforms are made of stainless steel, making debris on the scale easily visible and easy to clean. Unexpectedly, the scales from the display area showed

fairly high levels of 161 RLUs. Workers' negligence due to pressure from the management and customers could have attributed to these levels, compromising the sanitation in this butchery. The results suggest the need for a proper procedure and periodic cleaning to rid scales of lubricants and impurities (Toldrá, 2010).

The organic soil levels of butchery B are shown in Figure 6.3. Butchery B showed criteria of somewhat dirty with the ATP level ranging from >100 to <1000 RLU as well as group data standard deviation of STD_g=635.90. Bagge-Ravn *et al*. (2003), reports that microbial ecology reflecting the food preservation conditions vary according to each food processing premises. At a glance, butchery B appeared to adhere to the culture of food safety and the majority of the items were clean according to the recommended specification of ATP levels of >100 RLUs (Kaivac Cleaning Systems, 2011). Only two items (hooks and sinks) were considered filthy on a few sampling occasions, with ATP levels of >1000 RLU.

The high ATP levels, with an average of 1503 RLUs on hooks, might have been due to poor cleaning and sterilization practices. On observation, thermometers were never used for the reassurance of the sterilization of water of 82°C as recommended by the Department of Agriculture, South Africa (2007). Also, workers use the hooks to pull carcasses on the dressing line in the case of red meat, with the materials of overhead rails and supporting structures in the refrigerated rooms worn out and possibly contributing to the high levels. In addition, the hygiene of the hooks is significantly

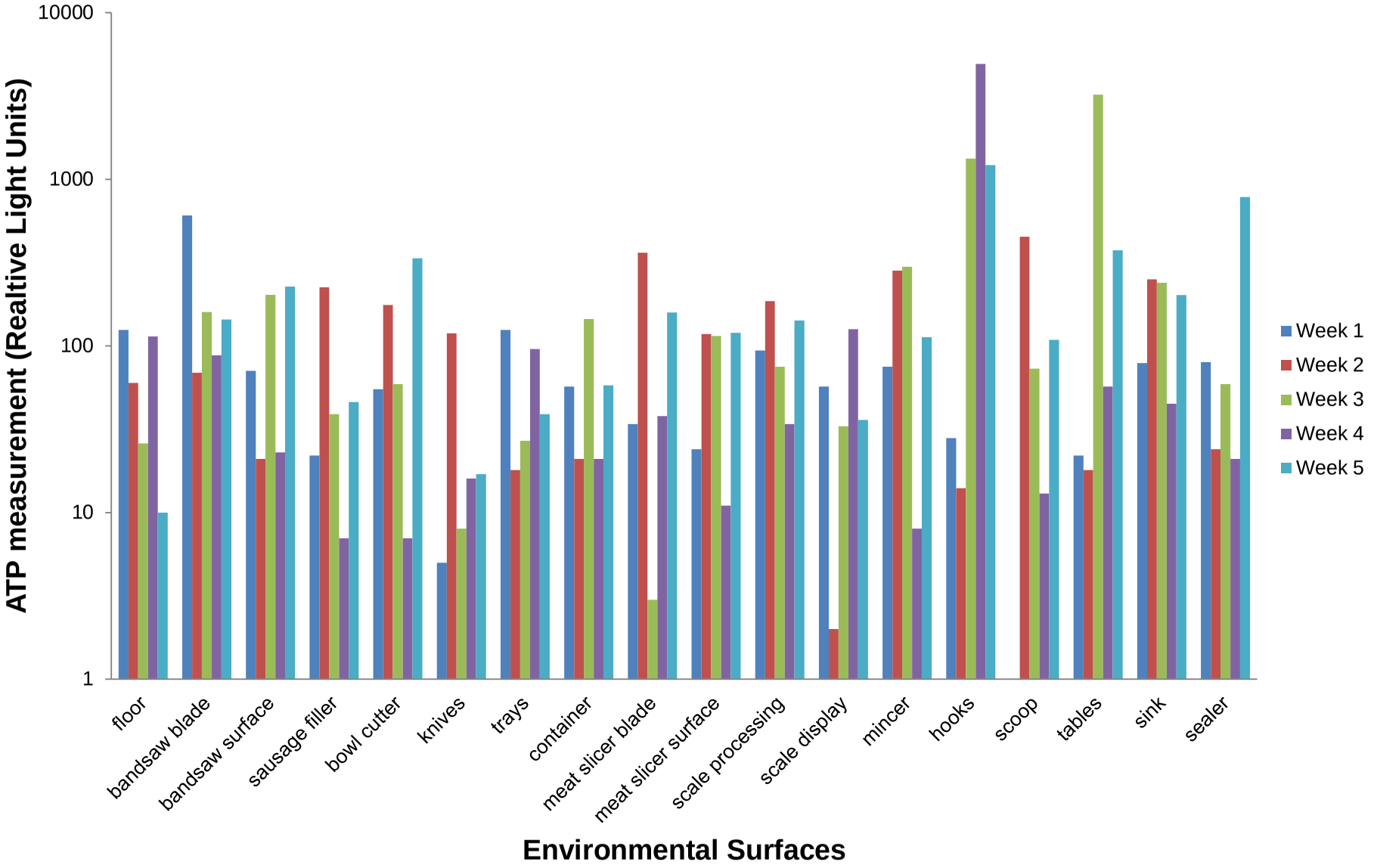

Figure 6.3: ATP assay on environmental surfaces at butchery B

affected by the design of the hooks, and also by the hygiene habits of the workers. Galvanized steel or food-grade aluminium is considered to be useful in meat processing materials for their non-corrosive properties (Heinz and Hautzinger, 2007).

The material of the overhead rails is not expected to contact meat directly as this may produce unwanted substances contaminating the hooks and meat products. It is the responsibility of the manager and workers to ensure that all equipment is effectively cleaned. Grbalová *et al*. (2003) suggests that equipment and utensils should be handled with care by avoiding the accumulation of organic matter, as even stainless steel may become uneven and rough.

High ATP values were found on trays in butchery C at an average of 2057.6 RLUs with group standard deviation at STD_g=1363.36. The lowest average of 55.-56.4 RLUs was shown on the meat slicer, designating good hygiene in this area (Figure 6.4). The results indicated that the material and the unsanitary conditions of the trays may reduce the shelf life of meat stored in the trays. In the study conducted by Mahdi *et al*. (2012), it was shown that the application of nano silver trays can enhance the quality and the shelf life of meat products. Hooks in the current study showed high contamination with an average of 1290 RLUs in butchery C. This was most probably due to workers' negligence and the material attached on the hooks which may have contained organic material (fat, proteins), inorganic material (residues of cleaning solutions) and microorganisms (Verran and Whitehead, 2006). An average of 1503.2 RLUs on scoops

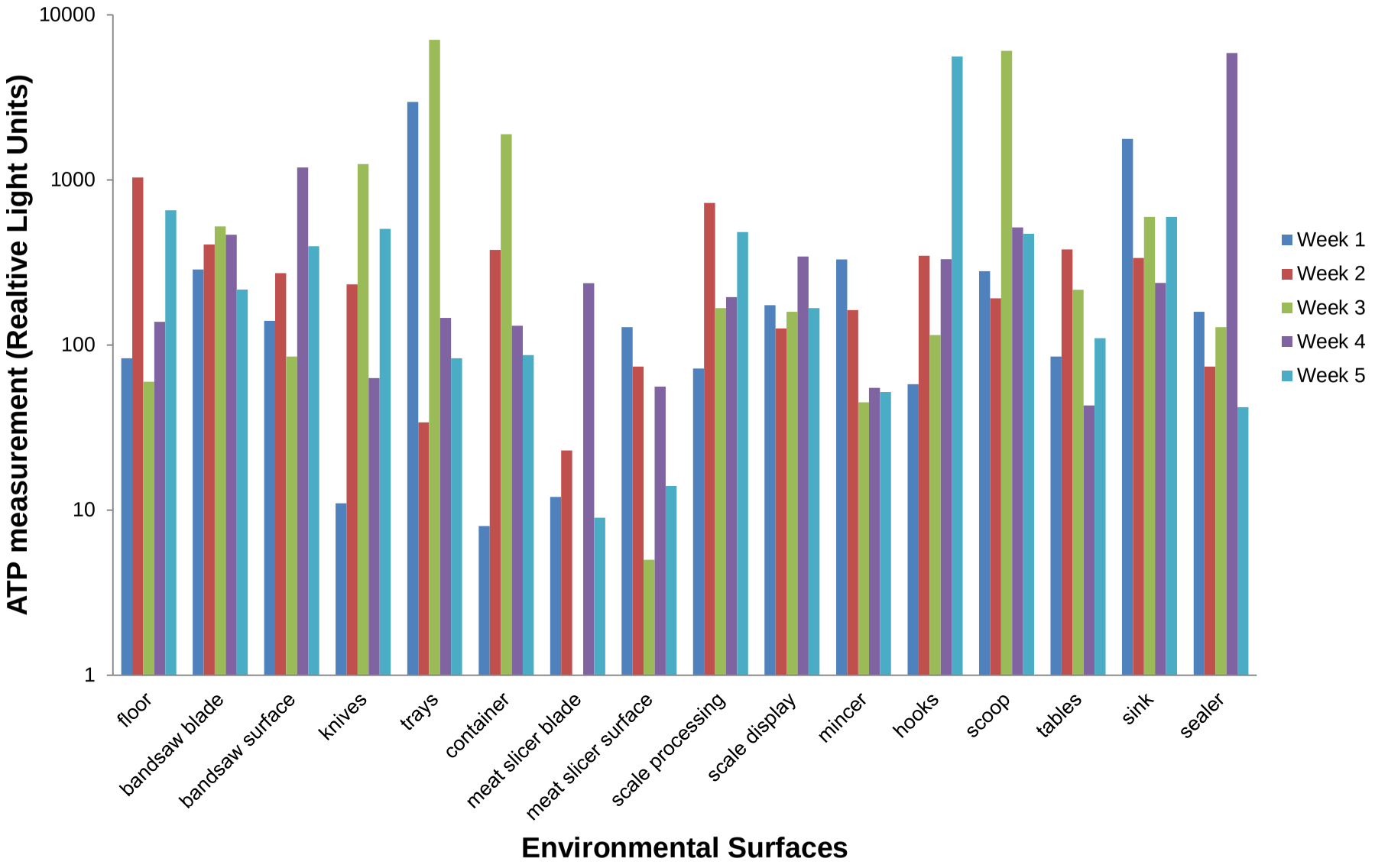

Figure 6.4: ATP assay on environmental surfaces at butchery C

was observed and this could be attributed to their corroded nature (they were chipped) which may hinder thorough cleaning and provides an environment conducive for the formation of biofilm. Environmental surfaces at meat processing establishments must be easy to clean, smooth, non-porous and free from cracks, crevices, scratches and pits which can possibly harbour and retain microorganisms and meat residues after cleaning (Hobbs and Roberts, 1993).

On the basis of visual observations and evidence of implementation of hazard analysis critical control point (HACCP) systems, butchery D was considered to be the cleanest of the butcheries studied. However, the RLU measurements suggested that even this butchery was not adequately cleaned as four items (meat container, meat mincer 2598.8 RLUs, sink average of 2072.8 RLUs and hooks 1034.8 RLUs, Figure 6.5) showed ATP levels of >1000 RLUs. The group data standard deviation was recorded at STD_g=1933.63 and the high ATP levels at this butchery suggested that the safety and quality of meat products were at risk, even though the establishment appeared clean. These results were in agreement with a study conducted by Moore and Griffiths (2002), where they reported that visual assessment can only reveal gross soiling but not smaller meat or food residues on the surfaces, or possible microbial contamination. Furthermore, several studies have shown that the RLU measurements can be greatly affected by chemical cleaning agents and commercial sanitizers (Green *et al*., 1998, 1999; Krysinski *et al*., 1992).

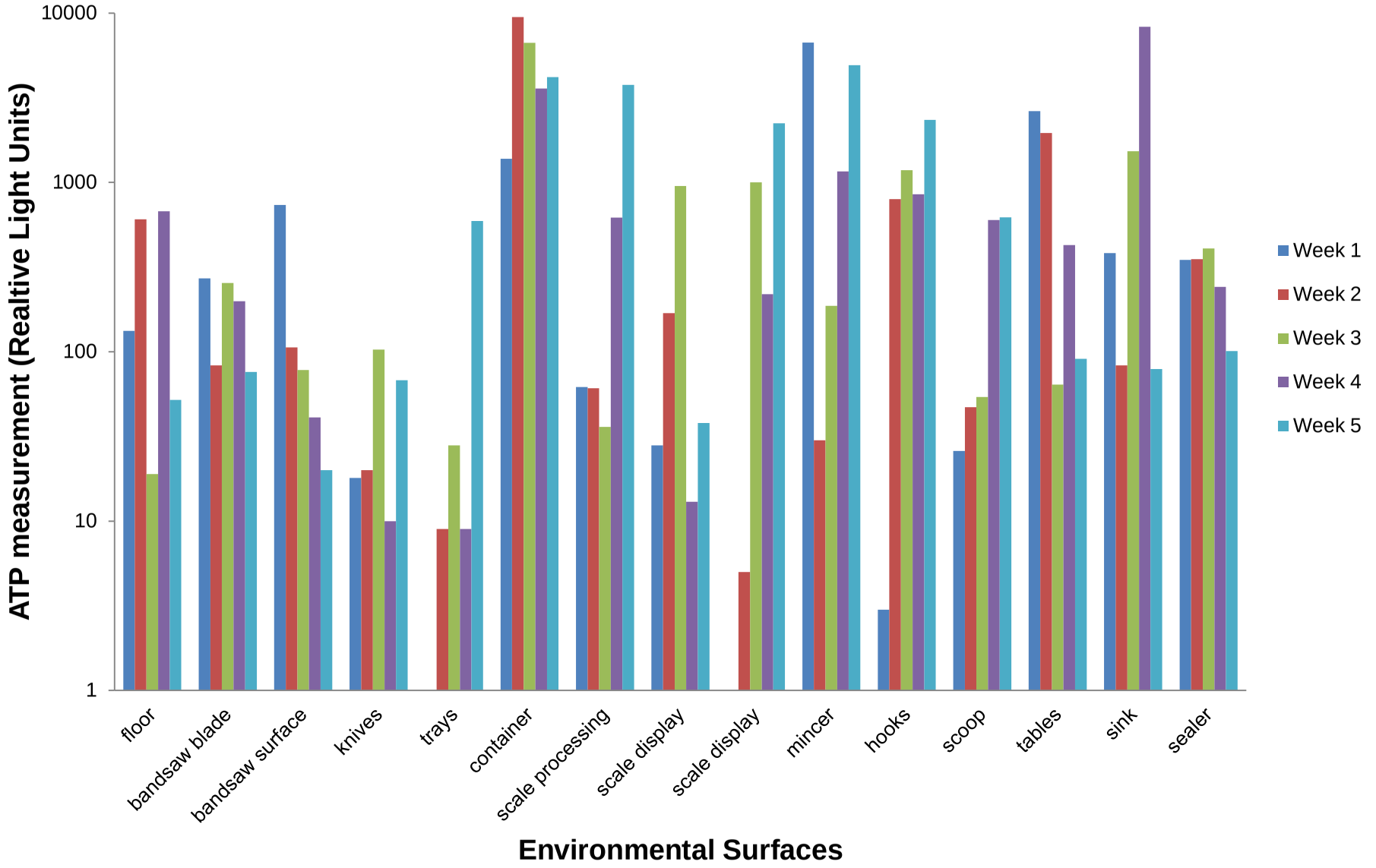

Figure 6.5: ATP assay on environmental surfaces at butchery D

Indirect food contact surfaces such as floors and sinks are often overlooked and are favourable environments for bacterial growth as they are commonly wet due to the activities taking place in butcheries (Buckalew *et al.,* 1996). In the current study, sinks were considered filthy with an average of 2072 RLUs. Similar results were found in a study conducted by Ojima *et al*. (2002) where sinks were found to be heavily contaminated with microorganisms.

In butchery E (Figure 6.6), the group data standard deviation was STD_g=873.62 with all items within the establishment having a considerably lower RLUs, ranging between ATP levels >100, but less than 1000 RLUs. However, hooks had the highest average of 1054 RLUs, which was still relatively low in comparison with butcheries A, B, C and D. Furthermore, tables showed ATP levels of 543 RLUs whilst sinks showed levels of 761 RLUs, which were higher than the ATP level of >100 RLU, indicating hygiene failure (Kaivac Cleaning Systems, 2011). This may be a possible source of microbiological contamination to meat and meat products during processing. According to Kusumaningrum *et al*. (2003), cutting equipment, hooks and tables are the crucial food contact surfaces in the food and meat industry and if contaminated they may pose a risk to food products. In the current study, the high ATP levels of hooks from all the butcheries suggested that they might be a real risk due to the persistence of organic soils which might be foodborne pathogens.

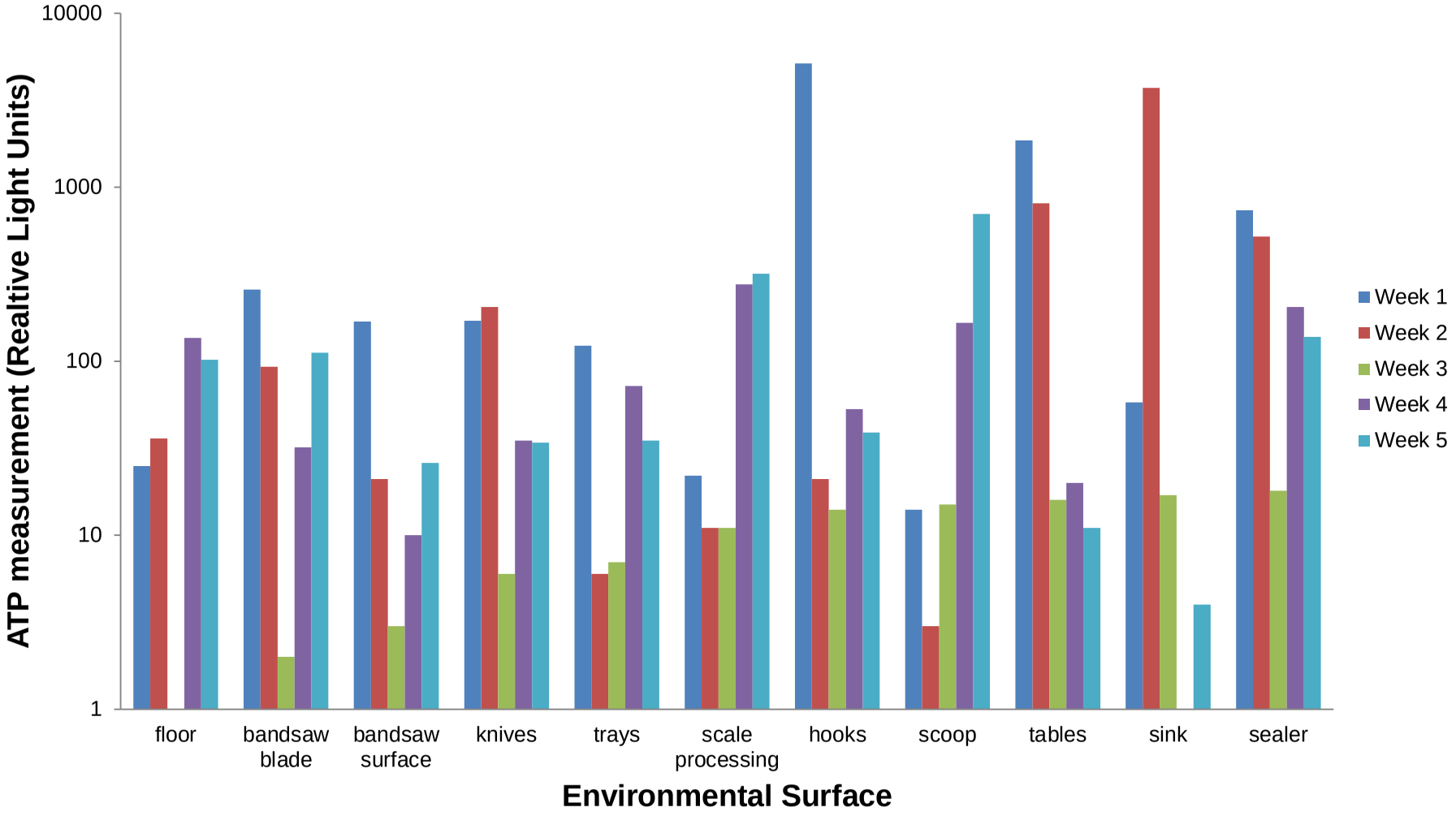

Figure 6.6: ATP assay on environmental surfaces at butchery E

In addition, Table 6.1 below reflect significant differences between butcheries over the entire sampling period with regard to ATP Hygiena RLU's quantified. Environmental monitoring in the meat processing establishments is aimed at assessing the industry's sanitation programmes and detecting organic soils including pathogenic and spoilage microbes affecting food safety. Thus, ATP in this study was found to be important in environmental sampling due to its ability to provide results that are reliable and accurate in real time for corrective action, possibly ensuring production of safe quality products. The current study provided evidence that high levels of RLUs on environmental surfaces were caused by a lack of monitoring and periodic verification checks as well as microbiological testing. Furthermore, limited environmental monitoring gave both food handlers and managers an impression that there was no problem. Hooks and sinks in all the butcheries were found to be heavily soiled indicating a possible source of contamination. This could be attributed to the presence of meat residues and/or microorganisms on the hooks as a result of the poor cleaning practices (Leon and Albrecht, 2007). During the processing of meat and meat products, sinks become an important source of contamination due to fat residue from a variety of carcasses.

6.5 Conclusions

In conclusion and a matter of recommendations, the ATP technique can be used for the education of workers handling meat, the managers and cleaning staff of the meat establishments as well as for their personal hygiene. Additionally, the studied butcheries should be encouraged to implement and maintain an HACCP system while also utilizing

Table: 6.1: Significant values for ATP Hygiena RLU's within selected butcheries.

Butcheries grouping	**Significant value (p)**
A and B	0.89
A and C	0.003
A and D	0.047
A and E	0.08
B and C	0.002
B and D	0.06
B and E	0.10
C and D	1.99×10^{-6}
C and E	0.0001
D and E	0.91

the ATP technique within the system as a monitoring tool. The results of this study further suggest that the current, common practice of evaluating food contact surface cleanliness visually by Environmental Health practitioner to meet the regulatory requirements might be inadequate. Thus, ATP bioluminescence operating capabilities proved to be an efficient tool to facilitate creation, implementation, and validation of more effective food contact surface cleaning and sanitation procedures in butcheries.

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CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

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7.1 SUMMATIVE REMARKS: HYGIENE PRACTICES OF MEAT HANDLERS IN MANGAUNG METROPOLITAN MUNICIPAL AREA BUTCHERIES

In this study, an attempt was made in Chapter 2 to evaluate the knowledge, attitudes, behaviour and general hygiene practices of meat handlers with the aid of a structured questionnaire. Additionally, personal observations, gained with the assistance of a checklist, were used to further report on practices of meat handlers during meat processing and the availability of infrastructure for food handlers to accomplish food safety. The overall results indicated a noticeable gap with regard to adequate training in the subjects critical to meat processing, handling and marketing. A key reason for this could be lack of management commitment and transparent communication in terms of food safety culture. Furthermore, the lower literacy rate and practical aspects of food microbiology played pivotal roles in achieving the results.

Some of the pressing concerns that emanated from the study included the lack of willingness of some managers to implement hazard analysis critical control point (HACCP) systems, which is known to be a pivotal aspect of a well-functioning food management system. It is evident that the poor reporting of illness and injuries was due to the drive to generate income in these selected butcheries. In the five butcheries, only two were found to adhere to good infrastructural development such as provision of hand-washing facilities and mechanical ventilation systems to control air. In addition, the same two butcheries had formal procedures for effective cleaning and employees could differentiate between words such cleaning and disinfection.

The respondents also indicated that they seldom had inspections, discussions or training from the environmental health practitioners regarding food safety issues. Hence, during observations most of the meat handlers were found to be wearing jewellery and had long to have long fingernails. In some instances, incidents occurred where female respondents were found to have worn make-up and Hair nets improperly. Although food safety is a shared responsibility amidst government departments, establishment managers and workers; food handlers' knowledge in personal and general hygiene plays a pivotal role in the prevention of cross-contamination. From the current study, it is evident that increasing statistical surveillance data in South Africa could promote effective food safety systems and empower those in charge with the required knowledge before incidents occur.

7.2 SUMMATIVE REMARKS: FINGER-PRINTING OF BIOAEROSOLS WITH MALDI-TOF MS

In Chapter 3 of this study, the distribution and occurrence of total viable counts was focused upon during meat processing in the different sections of the sampled butcheries. Different species enumerated were identified sequentially to determine their sources and their implications to human and the meat industry. Air samples were collected in four different sections of each establishment using 2 single surface air sampler (SAS-90) (PBI International, Milan, Italy).

The results indicate that total viable counts were generally higher in butchery D, in comparison with the results from related studies and butcheries A, B, C and E. However, an agreed microbiological guideline with regard to levels of airborne microbial counts in South Africa does not exist. Moreover, because meat processing is a labour-intensive activity, the high counts could have been generated during the processing activities as well as from poor plant layout that included a “braaing” facility. It was evident in this study that the number and behaviour of personnel greatly influences the generation of airborne microbes in food production areas (Schmitt, 2000).

The predominant Gram-negative bacteria isolated in the current study were members of the family *Enterobacteriaceae* and the Gram-positive airborne bacteria which included *Staphylococcus, Bacillus* and *Micrococcus* species. The isolation of these microbial organisms and other pathogenic species support the suggestion that bioaerosols can transport pathogenic and non-pathogenic bacteria through various routes, possibly contributing to the contamination of meat and meat products within the butcheries.

While this study established that there was no measurable relationship between aerial and carcass contamination, it clearly demonstrated that the air was an important source of microbial contamination including dangerous pathogens, highlighting the need for control measures to prevent airflow from dirty to clean areas.

7.3 SUMMATIVE REMARKS: BACTERIOLOGICAL ANALYSIS OF ENVIRONMENTAL SURFACES IN BUTCHERIES

The focus in Chapter 4 was on the hygiene levels of meat environment surfaces at selected butcheries in Mangaung Metropolitan municipal area which were evaluated using microbiological analysis. Although the meat contact surfaces conformed to the South African national standard of 1×10^2 cfu.cm^{-2}, the highest microbial load of 2.44×10^2 cfu.cm^{-2} was evident on the floor surfaces of all the butcheries. One possible explanation of this high level of contamination could have been due to the absence of a footbath at the entrance of the butcheries, as well as poor sanitary practices. The isolation of pathogenic organisms from the environmental surfaces was indicative of poor personal and general hygiene practices as well as an increased risk of cross-contamination in the meat processing area.

The results of the current study further suggest the importance of implementation, documentation, validation and on-going verification of HACCP for the production of wholesome meat products. Moreover, Environmental Health Practitioners should promote good health and hygiene practices at butcheries, utilizing bacteriological analysis and not only visual analysis. Meat handlers should be educated on food safety and the possible adverse effects (including their socio-economic impacts) of contaminated meat and meat products. Furthermore, the South African Department of Health, the Department of Agriculture: Veterinary Public Health, butchery owners and the media should introduce programmes that educate the public on the obligation of good sanitary practices and their possible effect on meat quality.

7.4 SUMMATIVE REMARKS: QUANTIFICATION OF MICROBIAL CONTAMINANTS ON HANDS AND APRONS OF MEAT HANDLERS IN BUTCHERIES

In Chapter 5 of this study, employee hygiene, as well as the occurrence of microorganisms on their hands and aprons, was determined. From the results of bacterial counts, the right hands of males showed the highest total viable counts of 3.42×10^{1} cfu.cm^{-2}. The highest total counts observed on females hands were 4.01×10^{1} cfu.cm^{-2}. The results of the present study showed higher levels in comparison to the results of a study conducted by Lues and Van Tonder (2007) which was performed at delicatessens, where counts on hands of food handlers ranged between 2 cfu.cm^{-2} and 1.3×10^{1} cfu.cm^{-2}. Moreover, male aprons appeared to be the filthiest overall, with the lowest counts of 1.22×10^{0} cfu.cm^{-2} and the highest counts of 20.5×10^{1} cfu.cm^{-2} observed. These results exceeded the suggested general microbial target value of <2.5 cfu.cm^{-2} post-washing (Moore and Griffith, 2002).

It is evident from these results that hand-washing procedures were not adequately performed by food handlers in the butcheries. Generally, the results of the current study underscore the significance of improving hand hygiene and sanitation practices particularly in butcheries, where highly perishable foods are handled and processed. Evidence from previous studies has demonstrated that microbial loads present on hands and protective clothing of food handlers can be reduced by washing hands and by the company providing clean protective clothing at the beginning of each working

shift (Martinez-Tomè *et al*., 2000; Tessi *et al.*, 2002; Michaels *et al.*, 2004; Regulation 918 (promulgated under the South African Health Act, 1977 and 1999)).

7.5 SUMMATIVE REMARKS: RAPID FOOD CONTACT SURFACE HYGIENE ANALYSIS USING ATP BIOLUMINESCENCE IN BUTCHERIES

In Chapter 6 of the current study, adenosine triphosphate (ATP) bioluminescence was used for the detection of organic soils on environmental surfaces in selected butcheries in the Mangaung Metropolitan municipal area. Organic soil levels found on hooks, sinks, floors and trays from all the butcheries were consistently higher than other environmental surfaces in the processing area. One possible explanation for these findings could have been due to the debris on surfaces, suggesting a lack of proper and regular cleaning. Moreover, this debris can result in the formation of biofilms, possibly resulting in the protection and proliferation of pathogenic microorganisms.

Unlike the case at abattoirs, where meat handlers entering and leaving the premises must pass through a disinfectant footbath placed at the entrance of the meat processing and slaughtering area, none of the butchery premises had this facility. The results of the current study were shared with the local municipality in order to enlighten them about the current hygiene status and the possible risks to public health. Moreover, Environmental Health Practitioners were advised to improve their inspection criteria at butchery premises where meat is processed and sold to the public. Additionally, butchery operators were advised to follow stringent health and hygiene practices

coupled with food safety training to improve the knowledge of meat handlers, possibly influencing their attitudes in terms of their practices and behaviour.

7.5.1 Comparative and statistical remarks on quantified counts

Table 7.1 below displays correlations coefficients between grouped sampled items per butchery. It can be noted from the table above that there was no statistical correlation between counts from ATP Hygiena and Total Viable Counts quantified from utensils and working surfaces. This is an indication that ATP Hygiena only focuses on debris and particulate matter which may not necessarily be of microbial origin but rather dust for example. Therefore, both methods must be used together to achieve the best results and most reliable.

7.5.2 Comparative microbial strains isolated

Extracting from chapter 3, the dominant isolated genera included *Bacillus, Kocuria, E. coli, Neisseria, Staphylococcus, Campylobacter and Pseudomonas*. The frequently isolated Gram-positive airborne bacteria included *Bacillus, Staphylococcus* and *Micrococcus* species which are known to cause spoilage in food. Gram-negative airborne bacteria isolated were mostly from the *Enterobacteriaceae* and *Pseudomonadaceae* families. Chapter 4 on the other hand, showed the most prominent microbial isolates identified in the study to be *Bacillus, Pseudomonas, Lactobacillus, Staphylococcus, Kocuria, Acinetobatcter, Micrococcus, Escherichia coli, and Neisseria*.

Table 7.1: Correlation coefficients of grouped items per butchery.

Butcheries	**A**	**B**	**C**	**D**	**E**
Coefficient values	0.189	0.047	-0.072	-0.231	-0.494

Lastly in chapter 5, the major bacterial pathogens isolated were *Escherichia coli, Staphylococcus aureus, Klebsiella spp., Pseudomonas aeruginosa* and *Bacillus cereus*, among others. Comparing these three microbial identification chapters it can be concluded that *Bacillus* was the most dominant species identified although this was not the case in chapter 5. Although other strains are not listed or not associated with food, their presence in the study is of great concern as some are associated with human health, animal origin and other potential sources not linked with food.

7.6 RECOMMENDATIONS TO THE BUTCHERIES

Although this study was only conducted in 5 butcheries representing only 15% of registered butcheries in the Mangaung Metropolitan Municipality, it is imperative to note that these results are likely to be similar in all butcheries country wide and possibly in the SADC region. It is thus crucial for the local government to make sure that all butcheries are registered. Secondly, the way the study was planned was more to solve the challenges faced by three of the non-conforming butcheries however, this data will be useful to all butcheries as the other two included butcheries which were conforming to the municipal requirements had similar challenges as other butcheries. Although training is a collective responsibility of managers and meat handlers in sustaining a culture of food safety, training programme evaluations should be conducted frequently to influence good hygiene behaviour. The benefit of formal training and motivation for a high standard of safe food handling with regard to the personal hygiene might be one key to safe meat processing in butcheries. Key personnel should be selected to perform

daily personal and general hygiene inspections since quality controllers are rarely available in butcheries. Respondents maintained that they had received food safety training; however, only a handful of them produced records as evidence. Therefore, record keeping should be emphasised for control and refresher training purposes as well as in case of disease outbreaks.

The realization that bioaerosols transport bacteria and contribute to the contamination of surfaces and also of processed meat with various implications validates the importance of monitoring airborne contamination in butcheries. Three traditional butcheries which showed high counts should pay attention to the improvement of the design of their buildings, as well as to proper ventilation to avoid bad odours and poor indoor air quality. Although it is impractical to expect to maintain the bacterial loads, yeasts and moulds at a zero level, ventilation systems in use should prevent air flowing from contaminated areas to clean areas so as to control microbial loads. Appropriate masks should be worn during the operations to prevent the creation and spread of airborne particles from workers coughing, sneezing and talking. Additionally, employees who are sick should be prohibited in the food processing area, rather should be booked off.

Effective cleaning and sanitation programmes should be implemented with the use of appropriate solutions (those that, in the form of aerosols or chemical residues, will not act as contaminants to food). Furthermore, the legal requirement clearly stipulates that at the start of each shift abattoir owners should provide clean protective clothing (Meat Safety Act 40, South Africa, 2000) this should also be implemented at the butcheries.

ATP bioluminescence in the butchery industry could be a remarkable educational tool used to measure the environmental surface cleanliness and effectiveness of sanitation procedures in real time. In addition, the ability of ATP bioluminescence portable machines to maintain data within the system could be useful for late analysis and verification.

7.7 RECOMMENDATIONS FOR THE MUNICIPALITY AND DEPARTMENT OF HEALTH

Health surveillance data is an important tool for public health interventions. However, in South Africa this is generally lacking, leading to uneducated personnel and maximizing the risks of foodborne illness to the public. From the current study, the use of MALDI-TOF MS for microbial analysis has shown that the pattern of microorganisms changes on an on-going basis with various health implications. Strengthening notification by means of a disease surveillance system may be a useful tool to guide policy makers in the Department of Health, for instance, for the benefit of public health.

As mentioned in the document, Environmental Health Practitioners rely mostly on visual assessment for the environmental inspection. It is therefore suggested that ATP bioluminescence should be utilized to inspect environmental surfaces as this can provide results rapidly, and could be ideal for the food processing industry where quality is important. Environmental Health Practitioners should emphasize the significance of good sanitary measures for the South African food business and consumer well-being.

It will also be crucial to have correlative work between local government and universities for research purposes. For an example, both parties could start looking into methods that are suitable for South Africa to assess contamination very fast in a form of biomarkers either microbial or particle contaminants. Furthermore, it must be mandatory for all butcheries to keep their cleaning schedules, medicals of all employees, their training needs and municipality must be involved during the training of meat handlers.

7.8 FUTURE RESEARCH

The results of the present study have indicated possible future research projects as follows:

- A study to determine the challenges that may be prohibiting the provision of a scientific and factual health surveillance data base.
- Assess the status of all buildings structures and air ventilation systems.
- Assessing the relationship between the microbes on environmental surfaces and meat processed in butcheries through the use of MALDI-TOF MS as an example.
- Conducting a similar study in other retail and traditional butcheries, in other provinces of South Africa.
- The effect of application of HACCP implementation systems in butcheries.

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APPENDIX A

A SURVEY OF HYGIENE PRACTICES OF MEAT HANDLERS IN BUTCHERIES IN THE MANGAUNG METROPOLITAN MUNICIPAL AREA, SOUTH AFRICA

QUESTIONNAIRE

SECTION A

DEMOGRAPHICS OF THE EMPLOYEES

1. Age range

18-21	22-30	Older than 40 years

2. The general education level

No education	Grade 6-9	Grade 12	Tertiary qualification

3. Duration of working period

Less than 3 months	3-12 months	More than a year

4. Employment status

Full-time	Contractor	Trainers	Seasonal

5. Race

Black	White	Indian	coloured

6. Gender

Female	Male

7. Hours of operation

Morning shift	Afternoon shift

8. Preferable language for food safety posters and trainings

English	Afrikaans	Sesotho	Xhosa

SECTION B

GENERAL HYGIENE PRACTICES

1. Personal hygiene	**True**	**False**	**Remarks**
Meat should be handled by persons with clean hands, fingernails and clothes			
Hands should be washed thoroughly with soap after every visit to a latrine			
Wounds, cuts and sores should be covered.			
Transmission of microorganisms from man to food occur through a carrier			
It is not important to wash hands frequently when you wear gloves			
Food handlers can wear jewellery in meat plant			
At least 20 seconds is enough for proper hand washing			
2. Protective clothing			
To prevent accumulation of bacteria washing and cleansing of steel mesh gloves should occur at regular intervals			
Gumboots should be washed frequently			
Protective clothing can be washed at home			
Clean protective clothing should be worn prior to the commencement of each shift			
Protective clothing worn by everyone entering processing including visitors area			
Hair and beard nets should be worn during meat handling			
3. Training and induction			
Provision of training to all the workers is important			
Appropriate skills and knowledge in food hygiene is essential in the food industry			
4. Transportation			
Cold chain maintenance ensures adequate shelf life and good quality meat			
Inspection of transport for cleanliness is important			
The vehicle transporting meat can also carry people			

5. Storage			
Meat storage room should be clean and sufficient to avoid cross contamination			
Different meat species (beef, mutton, chicken and pork) should be stored in a manner as to prevent cross contamination			
Raw meat cannot be stored with ready-to-eat meat			
Meat labelling is not important, looking at it one can tell which meat specie is			
One way to rotate meat products is to follow *first-in, first-out* (*FIFO*) *method*			
Food can be stored with chemicals as long as they are labelled			
Food products should be stored a minimum of 6 inches away from the wall and 6 inches away from the floor			
6. Receipt			
Specification plays an integral part in detecting health hazards.			
Inspection stamps indicate the raw meat received is from a licensed abattoir			
Inspection of vehicle for temperature and unwrapped meat is performed and valuable for this butchery			
Meat received is always free of contamination			
Frozen meat upon arrival is stored at -18ºC in 10min			
7. Refrigerator / freezer			
Refrigeration slows microbial growth			
Spoilage due to bone taint is unlikely to occur at 7°C			
Refrigerators are cleaned weekly			
Verification of the internal meat temperature is done with a use of a thermometer			
Maximum temperature for the dispatch of frozen meat is -12°C			
Cold rooms arranged in a first-in first-out basis			
8. Equipment			
Buildup of meat residues on meat cutting equipment can			

serve as a breeding place for insects and bacteria			
To prevent contamination all meat contact surfaces should be sanitised as often as necessary			
The hard to dissemble machine can be left out for inspection and the cleaning			
Contamination of surfaces/ products can occur due to buildup of or seepage of cleaning solvents			
Dead spaces in and around equipment can collect bacteria or insects			
Equipment must be cleaned, rinsed, sanitized and allowed to air dry			
9. Foodborne pathogens			
90% of bacteria can be removed by standard operating procedures (SOPs) in place			
Bacteria can be present on a sparkling clean surface			
Plant sanitation should be audited by an outside source such as a cleaning product supplier			
Cracked walls, floor and ceiling may harbour bacteria			
Some foodborne pathogens can survive in dry conditions			
Microorganisms cannot travel throughout the plant in water droplets generated by the use high pressure hoses			
Even healthy persons can harbour microorganisms in their (nose, hands, fingernails and on their skin)			
10. Plant sanitation			
Keeping the processing surfaces clean can reduce public health risks			
Clean walls and floors can only be identified visually			
Adequate lighting and ventilation should be provided throughout the facility			
Frequent removal of garbage is essential			
Colour coded brushes should be used			
11. Effective cleaning			
Cleaning schedule include detailed instructions for cleaning all areas of the facility			
A cleaning schedule should be used all the time			
Chemical manufacturer’s instructions should always be			

followed during cleaning			
Chemicals should be stored in a locked area			
There's a difference between cleaning and sanitizing			
Effectiveness of sanitizer is determined by the right proportion of (H_2O: sanitizer)			
Temperature of water important during cleaning.			
Read instructions before using chemicals			
12. Pest control			
Human hazard and precautionary statement appears on a label of pest control devices			
The devices are easily identifiable			
Rodents bait station tamper-resistant and secured to the ground			
Fly lights properly positioned			
Do you normally experience evidence of damage and debris caused by insects			

Appendix B

Appendix B:

Extra pictures showing sampled areas and/or items from various butcheries

Scheme 1. The above pictures show the floor with dirty bags and boxes thrown on the floor. The last picture reflects the condition of one of the working surface during the study.

Scheme 2. The above pictures reflect other surfaces and utensils used in some butcheries, mainly showing their status during working hours.

Scheme 3. The above pictures reflect one of the butcheries that performed very well with regard to cleaning procedures. Moreover, the pictures also highlight the used sanitizers which were lacking in some butcheries.

Scheme 4. The above pictures illustrate one of the butcheries with clean appearance as per their adherence to proper cleaning schedule and training of their workers.

Scheme 5. The above pictures illustrate two butcheries with one clean and the other used for braai purposes. This was one of the butcheries with lesser equipment's due to the nature of their operation.

Scheme 6. The above pictures shows comparison between two butcheries where bend saw was clean in the other and dirty in another butchery. One of the unclean butcheries is also reflected where quite a number of items were not properly packed, cleaned and processed.