A pilot study to evaluate the airborne bacterial load in veterinary surgical theatres without controlled ventilation systems

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Veterinary Industrial Pharmacology

By

Courtney van der Merwe

Department of Paraclinical Sciences

Faculty of Veterinary Science

University of Pretoria

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Supervisor: Professor Vinny Naidoo

I: DECLARATION

I declare that this dissertation, which I hereby submit for the degree Master in Veterinary Medicine in Veterinary Industrial Pharmacology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed: 

II: ETHICS STATEMENT

The author, whose name appears on the title page of this dissertation, has obtained, for the research described in this work, the applicable research ethics approval. The author declares that he/she has observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the Policy guidelines for responsible research.

Signed: 

III: DEDICATION

I dedicate this to my late dad, Johan van der Merwe, who awakened in me a passion for our four legged companions, thereby starting a journey that would lead me to the veterinary world.

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V: ABSTRACT

With an estimated 98% of the bacterial load in a clean surgical incision being derived from airborne pathogens, the bioaerosol composition of the theatre environment plays a determining role in the development of surgical site infections. From literature, focusing on human surgical theatres, it has been demonstrated that the concentration of viable airborne bacteria is influenced by the level of room occupancy, utilization of appropriate surgical attire and proper ventilation systems. Only limited information is however available for the veterinary profession.

This pilot study was aimed at evaluating the airborne bacterial load in veterinary practices which did not have the financial means for surgical theatre ventilation systems. Antimicrobial testing was furthermore performed to evaluate overall resistance of circulating bacteria in theatres.

Four veterinary facilities were recruited into the study. The sites differed in their surgical attire requirements, staff present during procedures and total daily throughput. As a way of quantifying the organisms that could settle in an incision, blood agar settle plates were placed within one meter of the incision site, from first incision to last suture, for routine canine or feline sterilizations. A total of 45 settle plates were collected and subjected to manual bacterial colony forming unit counts. Species were identified using the Sensititre ARIS 2x automated system; while antimicrobial susceptibility testing followed standard CLSI methodology.

After a total sampling time of 843 minutes, 487 bacterial isolates from 53 species were identified. *Micrococcus* (28.8%) and *Staphylococcus* (16.8%) represented nearly half of the isolates. A further 61.8% (24 species) could be classified as human and/or small animal commensals, with all but 4 being Gram-positive. Ten of the 53 isolated species (37.2% of isolates) were previously implicated in small animal surgical site infections (SSI's). Sensitivity testing was possible for 20.9% of the isolates representing 77.4% of the identified species. Resistance was detected in 58.8% of samples (80.5% of species). Of the organisms previously implicated in SSI's, 7 were tested for their antimicrobial susceptibility. *Micrococcus* isolates were resistant to kanamycin (7.1%), cephalothin (14.3%) and sulfisoxazole (28.6%). Trimethoprim/sulpha (20.0%) and erythromycin (20%) resistance was detected in

Staphylococcus pseudintermedius whilst *S. aureus* was susceptible to all antimicrobials. The single coagulase-negative *Staphylococcus* isolate was resistant to enrofloxacin, the *Pseudomonas* organism to cephalothin and *Enterococcus* to kanamycin, enrofloxacin, sulfisoxazole and tetracycline. Resistance to all 6 antimicrobials were detected in the single *Enterobacter* isolate.

Due to the limited sample size and variables between the facilities, it was not possible to make a statistically supported conclusion. Specific trends were however evident. A high level of room occupancy, lack of appropriate surgical attire, in conjunction with exposure to the outside environment, were factors associated with the high bacterial count. A correlation between the total occupancy time and commensal load was evident, with the higher throughput facilities yielding more commensal organisms per time period sampled, likely as a result of organism accumulation during consecutive procedures. For these reasons, it is concluded that current infection mitigation practices were not ideal to minimize the SSI risk. The routine wearing of correct attire and the implementation of routine cleaning procedures to reduce the bioburden for patient benefit are recommended.

VI: TABLE OF CONTENTS

VII: LIST OF FIGURES

VIII: LIST OF TABLES

IX: ABBREVIATIONS

AAMI	Association of the Advancement of Medical Instrumentation
AIA	American Institute of Architects
ACVIM	American College of Veterinary Internal Medicine
AMR	Antimicrobial Resistance
AORN	Association of periOperative Registered Nurses
ARG	Antimicrobial Resistance Genes
ASA	American Society of Anaesthesiologists
ASHP	American Society of Health-System Pharmacists
ASHRAE	American Society of Heating, Refrigeration and Air-conditioning Engineers
AST	Association of Surgical Technologists
ASTM	American Society of Testing and Materials
AVMA	American Veterinary Medical Association
BFE	Bacterial Filtration Efficiency
CDC	Centre for Disease Control and Prevention
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
CONS	Coagulase-negative *Staphylococcus*
COPS	Coagulase-positive *Staphylococcus*
DVTD	Department of Veterinary Tropical Diseases
ECC	*Enterobacter cloacae* complex
ESKAPE	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* species
FDA	United States Food and Drug Administration
FAO	Food and Agricultural Organization of the United Nations
GNID	Gram-negative Identification
GPID	Gram-positive Identification
HGT	Horizontal Gene Transfer
HVAC	Heating, Ventilation and Air Conditioning
HEPA	High-efficiency Particulate Air

IACG	Interagency coordination group on antimicrobial resistance
IDSA	Infectious Diseases Society of America
IM	Intramuscular
LBP	Lipopolysaccharide Binding Protein
LPS	Lipopolysaccharides
MGE	Mobile Genetic Elements
MRSA	Methicillin Resistant *Staphylococcus aureus*
MRSP	Methicillin Resistant *Staphylococcus pseudintermedius*
MSRAMMs	Microbial surface components recognizing adhesive matrix molecules
NICE	National Institute for Health Care and Excellence
NIOSH	National Institute of Occupational Safety and Health
NNIS	National Nosocomial Infections Surveillance System
OIE	World Organization for Animal Health
OR	Operating Room
PAMPs	Pathogen Associated Molecular Patterns
PBP	Penicillin Binding Proteins
PRR's	Pattern Recognition Receptors
SAL	Sterility Assurance Level
SAP	Surgical Antimicrobial Prophylaxis
SFM	Surgical Face Mask
SHEA	Society for Healthcare Epidemiology of America
SIS	Surgical Infection Society
Spp.	Species
SSI	Surgical Site Infection
WHO	World Health Organization

1. INTRODUCTION

Veterinary medicine is the branch of medicine dedicated to the promotion of health and the prevention of disease in animals. The surgical sterilization of canines and felines, which is among the most commonly performed procedures in small animal practice (Van Goethem et al., 2006, McKenzie, 2010), achieves this goal of safeguarding animal health by reducing the incidence of mammary tumours and eliminating the risk of pyometra in female patients, whilst decreasing the incidence of benign prostatic hyperplasia and prostatitis in males (McKenzie, 2010). The development of disease in the form of surgical site infections (SSI's), in what are healthy patients prior to the procedure, is therefore worrisome and fails to meet a key goal in the principle of 'do no harm'.

In its least severe form, SSI's result in delayed wound healing and increased patient morbidity; while in other cases leading to protracted hospital stays, secondary complications and even death (Nelson, 2011, Verwilghen and Singh, 2015, Darouiche, 2016, Badia et al., 2017). Because the human health care industry considers surgical site infections to be "the most common and costly of all hospital acquired infections" (Loyola-University-Health-System, 2007), surveillance systems, such as the Centre for Disease Control's (CDC) National Nosocomial Infections Surveillance System (NNIS), capture data related to these events (Weese, 2008). A total of 157 500 SSI's were reported in 2011 in the United States (Loyola-University-Health-System, 2007). Though a comparable reporting system is not available in the veterinary profession (Weese, 2008), it has been estimated that 2 to 6% of veterinary patients undergoing what can be classified as 'clean' or non-contaminated surgery, develop surgical site infections (Turk et al., 2015, Vasseur et al., 1988, Spohrc et al., 2012, Shales, 2012, Eugster et al., 2004). Considering the large number of operative procedures that are performed annually, the impact thereof is therefore likely as common in animals as in people (Nelson, 2011).

The development of a surgical site infection is dependent on the interplay between the degree of bacterial contamination, the virulence of the inoculating organism as well as the ability of the host's immune response to counteract or overcome this threat (Gawande et al., 2009, Nelson, 2011). The bacterial contamination involved with said infections can arise from one of

two sources, namely endogenously, referring to organisms which originated from within the body, either from sites of infection or from the normal inhabitants, or exogenously from the surrounding environment (Dixon, 1973, Owens and Stoessel, 2008, Burgess, 2019). In the case of a clean surgical procedure, as would be the case for a routine sterilization, it has been shown in human surgical facilities that approximately 98% of the bacterial load is derived from airborne pathogens (Whyte et al., 1982, Chauveaux, 2015). Thus, if the degree of airborne contamination can be controlled, the incidence of SSIs can be reduced.

The bioaerosol composition of a theatre environment is both complex and dynamic in nature. Through the shedding of squamous epithelial cells, hair and respiratory excretions, all occupants of the room contribute directly to the bioburden (Eugster et al., 2004, Srikanth et al., 2008, Weese, 2008, Al-Waked, 2010, Nelson, 2011, Trepanier, 2013, Adams et al., 2015, Barberán et al., 2015, Roy et al., 2018, Shaw et al., 2018, Singhal, 2018, Burgess, 2019). This correlation between the room occupancy and bacterial load is so strong, that it has been shown that each additional person increased the risk of surgical site infection 1.3 fold (Eugster et al., 2004, Trepanier, 2013). In order to 'contain' shedding, staff are therefore required to wear appropriate surgical attire which consists of masks, gloves, caps, scrub suits and gowns (FDA, 2019, Alexakis et al., 1976, Gawande et al., 2009, ASA, 2019a, AST, 2008). Despite these cautionary measures, it has been estimated that surgical staff can still shed approximately 10 000 squamous epithelial cells per person per minute, with 10% being expected to carry microorganisms (Al-Waked, 2010).

To further mitigate the continuous introduction of organisms into the environment, theatres can be equipped with heating ventilation and air conditioning systems (HVAC). These units not only serve to replace the volume of air in the operating room on a regular basis (Owens and Stoessel, 2008, Mangram et al., 1999, Singhal, 2018), but through the utilization of high efficiency particulate air (HEPA) filters, are able to remove particles larger than 0.3μm with an efficiency of 99.97% (Mangram et al., 1999, WHO, 2016, Shaw et al., 2018), irrespective of whether the air is recycled or introduced from the outside.

Despite the efficiency of modern ventilation systems, they only remove particles suspended in the air and thus, a thorough, systematic cleaning protocol which utilizes pre-approved disinfectants, is also needed to remove all settled particles and prevent the accumulation of organisms in the environment.

Even with proper surgical attire, ventilation and cleaning procedures, in some cases the inoculating bioburden is still enough to overwhelm the host's immune response, resulting in the development of surgical site infections. For this reason, prophylactic antimicrobial therapy, which aims to reduce the risk of SSI's, while at the same time minimizing the effect thereof on the patient's microbiota (Jocum, 2018), is commonly employed and often overused. Antimicrobial use in any form increases the selection pressure on both the target organisms as well as on the commensal bacteria (Weese et al., 2015). This leads to antimicrobial resistance, a problem so significant, that it was listed among the top 10 global health threats by the World Health Organization (WHO, 2019b).

While the bioaerosol composition of the theatre environment plays a crucial role in the development of SSI's (Sadrizadeh and Holmberg, 2015), the installation, running and maintenance costs of an appropriate ventilation system is usually not a financially viable option for the average veterinary practice. This can subsequently lead to veterinary theatres being contaminated with high bacterial loads, thereby necessitating the use of otherwise unnecessary prophylactic antimicrobials as a compensatory measure.

The aim of this study was to evaluate the airborne bacterial load present in veterinary theatres during a typical sterilization procedure, the possible factors that may have been contributing to the encountered bioaerosol composition, in addition to evaluating the degree of antimicrobial resistance in these organisms.

1.1 Hypothesis

Veterinary surgical theatres have low bioaerosol levels despite having no dedicated ventilation systems installed.

1.2 Study Aim

The aim of this pilot study was to identify the bacterial species that most commonly form part of the bioaerosol load in non-environmentally controlled veterinary theatres (i.e. theatres lacking a HVAC system with HEPA filtration) during routine canine and feline sterilizations.

1.3 Objectives

1. Identify the organisms that could potentially cause surgical site infections in a non-environmentally controlled theatre set up by placing settle plates around the surgical field.
2. Determine the antimicrobial susceptibility of the identified organisms
3. Evaluate current antimicrobial use practices employed by the participating facilities

2. LITERATURE REVIEW

2.1 Development of modern day surgery

Part of the deepest essence of man is his instinct for self-preservation (Aggarwal, 2010, Cherry, 2020), and therefore, in response to the near constant onslaught of disease and injury, which can be considered inseparable companions of life (Bishop, 1995), he has dedicated time, energy and countless resources to developing what is today known as modern medicine.

Attaining the level of care that is today considered standard did not, however, come without huge sacrifice. As recently as the 19th century, the odds were greater that a patient would die, rather than survive a surgical intervention. According to a publication on the first reliable operative statistics in 1841 (Sabbatani et al., 2016), it was reported that surgical amputation was associated with a staggering 60% mortality rate, primarily as a result of 'hospital diseases' (Sabbatani et al., 2016).

At the time, pus and suppuration were considered a normal part of healing (Sabbatani et al., 2016, Eschner, 2017), and the germ theory of disease, which states that disease can be caused by invasion of the body by micro-organisms (Rogers, 2019), was still simply a theory (Eschner, 2017). Consequently, it was not uncommon for surgical procedures to take place in unsanitary conditions. Operating rooms were described as having "faeces, urine, blood, and pus on the floors and sputum clinging to the walls" with sawdust being used to "absorb spilled blood and pus" (Smith et al., 2012). The surgical staff regularly donned gowns encrusted with dried bodily fluids (Sabbatani et al., 2016) and used instruments which were often not cleaned until put away for storage (Eschner, 2017). These atrocious conditions resulted in James Young Simpson (1811-1870), a surgeon at the time, commenting that "the patient lying on our operating room table has a greater risk of death than an English soldier on the battlefield in Waterloo!" (Sabbatani et al., 2016).

In 1867 Joseph Lister, who would later be dubbed as 'the father of antiseptic surgery' published a series of articles which would have a profound effect on the future of medicine (Wills, 2017). Lister, who believed that wound suppuration was caused by germs which were suspended in the air (Sabbatani et al., 2016), started using carbolic acid as a germicide (Eschner, 2017). The

concept of antisepsis expanded, eventually leading to disinfection of not only wounds, but also surgical instruments and the surgeons' hands. In some cases carbolic acid spray was even used to decrease the airborne load (Sabbatani et al., 2016, Smith et al., 2012). Though initially treated with scepticism, the results were profound. In 1881 a clinic in Basel Switzerland reported that mortality rates had declined from 77.7% to 10.2% in hernia repairs, 52.7% to 10% in open fractures and 43.7% to 11.5% in cases of amputation over the preceding 10 years (Sabbatani et al., 2016).

Carbolic acid was soon replaced with other antiseptics but the principles remained the same (Eschner, 2017). By the early 1900's, theatres were being thoroughly cleaned between procedures, surgical packs were sterilized and surgical attire, including gowns, masks and gloves, started gaining popularity (Smith et al., 2012). Over the next 100 years aseptic practices were refined, highly efficient ventilation systems were introduced and antimicrobial drugs were developed – measures which all served to further minimize the degree of bacterial contamination (Gawande et al., 2009).

Despite these advances, surgical site infections (SSI's) still have a staggering impact on today's health care industry – not only for the medical profession, but importantly the veterinary profession as well (Badia et al., 2017, Nelson, 2011).

2.2 Impact of surgical site infections

The United States Centre for Disease Control and Prevention (CDC) has defined surgical site infections as "infections of the incision or organ or space that occur after surgery" (Berríos-Torres et al., 2017). Depending on the severity thereof, these infections can significantly alter the quality of life of affected patients (CDC, 2019b). Delayed wound healing, protracted hospital stays, increased secondary complication rates and increased morbidity are among the commonly associated consequences. These infections are furthermore associated with an increased mortality rate (Darouiche, 2016, Badia et al., 2017). Apart from the direct consequences to the affected patient, surgical site infections place a significant financial burden on the health care industry resulting in additional expenses of approximately $3.3 billion per annum (CDC, 2019a). With surgical site infections accounting for 20% of all nosocomial infections in the human health care industry (Owens and Stoessel, 2008, Loyola-University-

Health-System, 2007), it is considered to be the "most common and costly of all hospital-acquired infections" (Loyola-University-Health-System, 2007).

Because of the significant impact of surgical site infections, both active and passive surveillance systems which capture data related to these events, have been developed (Weese, 2008). The CDC for example has the National Nosocomial Infections Surveillance System (NNIS) which, in 2011, recorded 157 500 surgical site infections in the United States alone (CDC, 2019a).

Though comparable reporting systems are lacking in the veterinary profession (Weese, 2008), the impact of surgical site infections are believed to be no less severe (Nelson, 2011). Depending on the source and the type of surgery, surgical site infection rates have been reported to affect anywhere from 2.0 to 28% of veterinary surgical patients (Turk et al., 2015, Nelson, 2011, Vasseur et al., 1988, Shales, 2012, Spohrc et al., 2012). While these figures may seem inconsequential, when one considers the millions of operative procedures performed annually on a global scale, the figures immediately represent a significant number of affected animals.

In order to effectively mitigate these staggering figures, it is important to first understand how surgical site infections develop.

2.3 Pathogenesis of surgical site infections

A number of factors play an important role in establishment of surgical site infections. These are detailed below.

2.3.1 Sources of bacteria

As implied by the term 'surgical site infection', bacterial contamination is a prerequisite for the development thereof (Gawande et al., 2009, Mangram et al., 1999). These intra-operative surgical site contaminants can be divided into two categories, namely those originating from endogenous sources and those that are derived exogenously (Dixon, 1973). Endogenous bacteria are derived from sites of infection or from the normal inhabitants that colonize the skin, nasal cavities or hollow viscera of the patient. Exogenous bacteria on the other hand, being derived from external sources, are influenced by the surgical personnel as well as by the theatre environment itself (Burgess, 2019, Owens and Stoessel, 2008).

2.3.2 Host immune response

Contamination alone does not necessarily equate to infection as the innate host resistance plays a vital role in protecting the body against foreign invaders (Tizard, 2013). Upon penetration of the body's physical barriers, pattern recognition receptors (PRR's) (i.e. proteins that are capable of recognizing molecules frequently associated with microbial invaders, namely pathogen associated molecular patterns or PAMPs), release signals that in turn trigger microbicidal and pro-inflammatory responses (Amarante-Mendes et al., 2018, Tizard, 2013). This rapid response by the innate immune system aims to contain, if not eliminate, the pathogen through phagocytosis or antibacterial exudates, whilst at the same time triggering the adaptive immune response (Amarante-Mendes et al., 2018, Tizard, 2013).

Once triggered, the adaptive immune response is able to combat bacterial infections through five main mechanisms (Tizard, 2013):

- Direct killing of bacteria by cytotoxic T and natural killer cells
- Phagocytosis of bacteria following their opsonisation
- Killing of bacteria by the complement pathway
- Destruction of intracellular bacteria by activated macrophages
- Binding of antibodies to toxins which prevent binding to target cells, thus neutralizing the toxin (Tizard, 2013)

Thus, in cases where it is adequate, the host immune response may prevent the progression to infection where bacterial inoculation into the surgical site has taken place (Singhal, 2018).

2.3.3 Physical status of the patient

The Physical Status Classification System, created by the American Society of Anaesthesiology (ASA), classifies patients according to their pre-operative physical status. Though originally developed for other purposes (Spohrc et al., 2012), this ranking system, which takes into account the degree of a patient's systemic disease (Table 2-1), has importantly been shown to correlate with the risk of surgical site infections in humans (Nelson, 2011).

Table 2-1: America Society of Anaesthesiology physical status classification system

(Daabiss, 2011, ASA, 2019b, Mayhew et al., 2019)

ASA score	Definition

1	A patient that is completely healthy
2	A patient that only has mild systemic disease
3	Severe systemic disease is present in the patient, but it is not yet incapacitating
4	The severe systemic disease is a constant threat to the life of the patient
5	The patient is not expected to survive without the surgery (i.e. moribund)

2.3.4 Organism virulence

Though the likelihood of an infection developing may be smaller, a normal healthy patient is not free from risk. The inherent virulence of the inoculating organism greatly affects not only its ability to survive, but also to thrive and replicate in spite of the host's immune response (Mangram et al., 1999, Peterson, 1996). The virulence tactics employed by respective bacteria vary greatly with some organisms concealing themselves from the immune response, others blocking or disrupting the response once present, while others actually trigger and escalate the immune response with resultant greater tissue damage (Gawande et al., 2009, Mangram et al., 1999). Ultimately, the more virulent the organism, the more likely it is to cause a surgical site infection (Gawande et al., 2009).

Some common examples are highlighted below:

- The *Pseudomonas* species, which has been implicated in canine and feline SSI's, has the ability to form a biofilm. This 'mucoid-like' layer consisting of various extracellular polymeric substances not only supports growth by allowing for substrate exchange and distribution of metabolic products between organisms, but it importantly provides protection against the host immune response by resisting phagocytosis (Foster et al., 2014, Birte Hollmann et al., N.D.)

- As with a biofilm, the bacterial capsule provides a defensive shield, protecting the organism from host immune recognition (Wen and Zhang, 2015). Unlike biofilms which are released into a matrix and thus only loosely attached to the cell wall, the thick, compact bacterial capsules are physically associated with the cell and can thus not be washed off (Wen and Zhang, 2015, Kandi, 2015). The hydrophobicity and negative charge of these polysaccharide structures hinders phagocytic killing (Gawande et al., 2009, Zhensong and Zhang, 2015) an important early step in host response (Gawande et al., 2009). This

important structure thereby assists SSI-causing organisms such as *Pasteurella multocida* and some *Streptococcus* species (Gawande et al., 2009, Burgess, 2019, Verwilghen and Singh, 2015, Turk et al., 2015) in evading the immune response (Wen and Zhang, 2015).

- Unlike a capsule and biofilm which helps the organism to evade the immune response, lipopolysaccharides (LPS), contained in the outer membrane of most Gram-negative organisms, including *Pasteurella multocida,* are a primary stimulator of the host immune response (Harper and Boyce, 2017, Sampath, 2018). Despite consisting of multiple components, it is lipid A, the membrane anchoring and endotoxic component, which strongly stimulates both the innate and adaptive immune responses (Harper and Boyce, 2017, Sampath, 2018). By binding to lipopolysaccharide binding protein (LBP), a plasma protein, lipid A, sets the immune cascade in motion (Harper and Boyce, 2017). A simplified version of this process is portrayed in Figure 2-1 below.

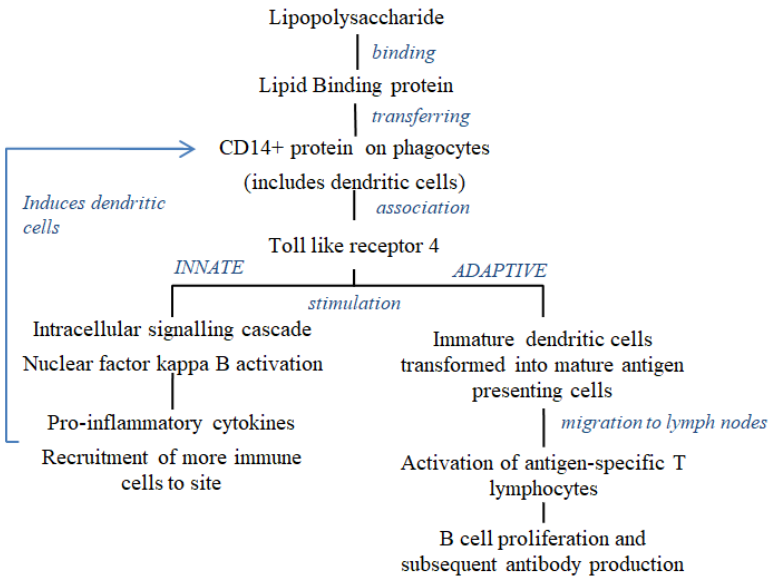

Figure 2-1 Activation of the immune system following lipopolysaccharide signalling

(Harper and Boyce, 2017, Sampath, 2018)

The most successful organisms however often utilize a combination of virulence factors to establish themselves. The normal canine skin commensal, *Staphylococcus pseudintermedius*, also known as 'dog's golden staph', is a prime example of this. Host colonization, which is a prerequisite for the establishment of infection, is mediated by a group of microbial surface proteins known as microbial surface components recognizing adhesive matrix molecules (MSRAMMs). As implied by the name, these proteins have the ability to recognize and bind to various host surface proteins (Foster et al., 2014, Maali et al., 2018). Various proteolytic enzymes, including, but not limited to, coagulase and von Willebrand factor binding protein, further aid the colonization process (Maali et al., 2018, Wladyka et al., 2015). Once established, dissemination is accomplished by modulation of the immune response, through hemolysins, leucotoxins and chemotaxis inhibitory proteins, thereby overcoming competitive bacteria (Wladyka et al., 2015). This combination of virulence factors, in conjunction with its commensal relationship with the host, has allowed *S. pseudintermedius*, which is considered the canine equivalent of *Staphylococcus aureus* infection in humans (Wladyka et al., 2015), to become the "most common cause of canine SSI worldwide" (Verwilghen and Singh, 2015).

Additional examples of bacterial pathogens which have been implicated in canine and feline surgical site infections include, enterococci, coagulase-negative staphylococci, and extended

spectrum beta-lactamase producing *Enterobacteriaceae* (Gawande et al., 2009, Burgess, 2019, Verwilghen and Singh, 2015, Turk et al., 2015).

2.3.5 The dynamic nature of SSI's

As has been explained above, the development of a surgical site infection is dependent on the dynamic relationship between the degree of bacterial contamination, the virulence of these organisms and the ability of the host to counteract these organisms (namely the host defence) This relationship can be summarized as follows: (Gawande et al., 2009, Nelson, 2011)

$$Surgical\ site\ infection\ risk = \frac{bacterial\ contamination\ \text{X}\ bacterial\ virulence}{host\ resistance}$$

Of the above mentioned factors, the degree of bacterial contamination is the factor which can most easily and reliably be controlled. It is for this reason that surgical site infection mitigation measures place emphasis on decreasing the degree of contamination at variable points as a means of reducing surgical site infections.

2.4 Controlling bacterial contamination

2.4.1 Correlation between bacterial contamination and procedure type

Procedures can be divided into 1) clean (in which no hollow viscera is entered and there is no break in aseptic technique), 2) clean-contaminated (in which hollow viscera is entered but there is no subsequent spillage), 3) contaminated (introduction of bacteria into normally sterile areas) and 4) dirty (in which active infection is established) (Weese, 2008, Nelson, 2011, Vasseur et al., 1988). As can be derived from the classification system, bacterial loads are expected to increase moving from clean to more dirty procedures. The source of infection in clean procedures usually originates outside of the surgical field, whilst clean-contaminated, contaminated and dirty procedures on the other hand are associated with increasing endogenous bacterial loads and consequently higher SSI rates (Nelson, 2011), as can be seen in Table 2-2 below.

Table 2-2 Veterinary surgical site infection rates reported in various publications categorized according to procedure type

Surgical Site Infections (%)				
Clean	**Clean Contaminated**	**Contaminated**	**Dirty**	**Reference**
3.2	3.8	0	20	(Turk et al., 2015)
2.5	4.5	5.8	18.1	(Vasseur et al., 1988)
2.5-6	2.5-9.5	5.8-28	18-25	(Spohrc et al., 2012)
2-5[a]		10-12[b]		(Shales, 2012)
2.0-4.9	3.5-4.5	4.6-9.1	6.7-17.8	(Eugster et al., 2004)

[a] No distinction was made between clean and clean contaminated procedures
[b] No distinction was made between contaminated and dirty procedures

2.4.2 Endogenous pathogens

Endogenous pathogens arise mainly from the patient's skin, mucous membranes, and except in the case of surgeries classified as clean, also from hollow viscera (Gawande et al., 2009). Of these, organisms located on the skin are those which are most easily controlled.

An intact epidermis provides a protective barrier against pathogens. Any disruption to this barrier can serve as a potential point of entry for these endogenous pathogens (Coates et al., 2018). For this reason, skin preparation should be done in a manner which does not compromise skin integrity (Griffin et al., 2016). Due to the micro-abrasions caused by shaving, the World Health Organization (WHO) does not recommend hair removal prior to surgery unless it interferes with the surgical site (Gawande et al., 2009). In veterinary practice however, where patients have more hair, hair removal is considered standard procedure with the recommendation being that it should be done in a manner which minimizes trauma. Clipping is therefore recommended over shaving (Gawande et al., 2009, Mangram et al., 1999, Verwilghen and Singh, 2015). The reason for this is that the microscopic abrasions caused by shaving create foci for bacterial multiplication, in turn resulting in increased surgical site infection rates (Mangram et al., 1999). Hair removal should furthermore be done as close to the start of the procedure as possible (i.e. after induction), as this minimizes the time which bacteria have to colonize the area thereby reducing the incidence of surgical site infections (Weese, 2008, Gawande et al., 2009, Verwilghen and Singh, 2015, Trepanier, 2013).

Though not sufficient to completely eliminate endogenous skin microflora, adequate preoperative skin preparation can greatly reduce the number of these organisms (Gawande et al., 2009). The skin disinfectant used should, according to the Food and Drug administration, be "fast acting, broad spectrum and persistent" and should additionally "significantly reduce the number of microorganisms on intact skin" (Gawande et al., 2009). Some examples of appropriate agents include chlorhexidine, iodine, alcohol and triclosan based products (Mangram et al., 1999). The product should furthermore be applied in outwardly radiating concentric circles, moving from the incision site to the periphery (Gawande et al., 2009, Mangram et al., 1999).

2.4.3 Exogenous bacteria

Utilizing a combination of wound washout, patient skin sampling, settle plates and volumetric air sampling during 52 hip and knee arthroplasties, Whyte et al. (1982) was able to determine that, in a conventionally ventilated operating room, approximately 98% of the bacterial load in a clean surgical incision site is derived from airborne pathogens and that the rate at which airborne bacteria contaminate the wound is directly proportional to the airborne count (Whyte et al., 1982). This is in line with the statement made by Sadrizadeh and Holmberg (2015) in which they state that "the infection risk of surgical patient is significantly correlated with the concentration of viable airborne bacteria".

Therefore, based on current knowledge, air is considered the most important exogenous source of bacterial contamination (Chauveaux, 2015). More importantly, the concentration of viable airborne bacteria is directly influenced by level of occupancy of the room (Weese, 2008, Burgess, 2019, Roy et al., 2018, Shaw et al., 2018, Barberán et al., 2015, Al-Waked, 2010, Srikanth et al., 2008, Adams et al., 2015, Nelson, 2011, Trepanier, 2013, Singhal, 2018).
It is estimated that each person, in addition to their respiratory excretions, sheds approximately 30 000 to 40 000 dead skin cells per minute (Sandle, 2014, Prussin and Marr, 2015). Appropriate attention should therefore be given to limiting the degree of airborne bacterial contamination. Among these mitigation measures would be the utilization of appropriate surgical attire by the staff members in the theatre (Alexakis et al., 1976). In a study which evaluated the factors influencing microbial colonies in the air of operating rooms, Shaw et al. (2018) found that each additional staff member increased the bacterial colony counts by

4.93cfu/m^3. This direct correlation between the number of surgical personnel and the degree of airborne bacterial contamination (Shaw et al., 2018) has been mirrored in veterinary research (Weese, 2008) where it was found that each additional person increased the risk of surgical site infection by 1.3 times (Eugster et al., 2004, Trepanier, 2013). For these reasons it is logical that only essential personnel should occupy surgical theatres, and that movement within and into and out of theatres, should be kept to a minimum (Shaw et al., 2018, Nelson, 2011).

2.4.3.1 Appropriate surgical attire

Standard surgical attire is currently comprised of a surgical gown, mask, scrub cap and sterile gloves (AST, 2008, Gawande et al., 2009, ASA, 2019a, FDA, 2019). These items serve to protect not only the surgical staff, but importantly also the patient, from the transfer of micro-organisms, body fluids and particulate matter (FDA, 2019).

Caps: The importance of caps was highlighted circa 1985/88 when an outbreak of *Streptococcus* group A surgical site infections involving 20 patients was directly linked back to the scalp of a single surgical staff member (Mastro et al., 1990). Despite no reported outbreaks since then (to our knowledge), the scalp of a person, which is colonized with approximately 1 000 000 organisms per cm^2 (Sandle, 2014), could still potentially serve as a source of micro-organisms. Surgical caps, which contain hair and bacteria which would otherwise contaminate the sterile field, are thus considered part of standard surgical attire (Gawande et al., 2009, Owens and Stoessel, 2008, Roy et al., 2018, Mangram et al., 1999, Singhal, 2018). Despite the recommendation for their use, following an extensive literature review, AORN (Association of periOperative Registered Nurses) was unable to find any association between type of material and degree of hair coverage and SSI rates. They were thus unable to provide further specific recommendations apart from the removal of surgical caps at the end of a shift or earlier if they are contaminated (AORN, 2019).

Masks: Masks have been shown to significantly decrease tissue contamination (Roy et al., 2018, Mangram et al., 1999). In order to achieve this, the material from which the surgical face mask (SFM) is produced, must provide a sufficient barrier to

biological contaminants whilst at the same time minimizing condensation and moisture build up within the mask (Datta, 2010). Industry standards, as recommended by The American Society of Testing and Materials (ASTM), require that masks conform to certain specifications including but not limited to their Bacterial Filtration Efficiency (BFE), Particulate Filtration Efficiency and Separating Efficiency (Datta, 2010). Though not directly linked with a reduced incidence of surgical site infections a SFM, which covers both the mouth and nose is nonetheless considered to be a standard component of surgical attire by the World Health Organization and CDC (Gawande et al., 2009, Owens and Stoessel, 2008).

Gloves: According to World Health Organization guidelines regarding hand hygiene in health care, bacterial counts on the hands of health care workers range from 3.9 $x10^4$ to 4.6 $x10^6$ colony forming units per cm^2 (WHO, 2009), with the potential existing for these organisms to cause infection in sterile body cavities (WHO, 2009). Appropriate hand hygiene (as takes place with a pre-surgical scrub) can serve to reduce contamination, with the efficacy thereof being dependant on both the preparation used, as well as the duration of application (WHO, 2009). Alcohols for example, which have the ability to denature proteins, have a rapid onset of action (Hsieh et al., 2006), reducing the release of test bacteria from artificially contaminated hands by 4.0-5.0 $\log_{10}$ after a 1 minute application (WHO, 2009).

Despite its ability to reduce bacterial contamination, alcohol is not able to eliminate all organisms. The same holds true for chlorhexidine, iodine/iodophors, triclosan and parachlorometaxylenol, other commonly used antiseptic agents (Hsieh et al., 2006). Intact surgical gloves serve as a barrier, thereby preventing the spread of pathogens from health care staff (Hsieh et al., 2006). Apart from the tensile and barrier, integrity, elongation and leak standards that sterile gloves should meet (FDA, 2008), the United States Food and Drug Administration (FDA) requires a sterility assurance level (SAL) of 10^{-6} for surgeons gloves (FDA, 2008) i.e. “a probability of not more than one viable microorganism in an amount of one million sterilised items of the final product” (von Woedtke and Kramer, 2008), thereby ensuring that an intact surgical glove is not further contributing to the

bioburden. Though the benefit of sterile surgical gloves have not been quantified due to the ethical concerns of conducting a study where the control group is lacking adequate hand protection, historical evidence is sufficiently strong that sterile glove use is highly recommended by the CDC (Owens and Stoessel, 2008) and consequently considered a standard part of theatre attire (Gawande et al., 2009, Burgess, 2019, Owens and Stoessel, 2008, Roy et al., 2018).

Gowns: Surgical gowns which cover a large portion of exposed skin are worn in the operating room to contain shed skin cells and microbes (Hee et al., 2014, FDA, 2019). Additionally, because organisms are able to penetrate a material when wet, surgical gowns must conform to manufacturing and testing standards as set out by the AAMI (Association of the Advancement of Medical Instrumentation (CardinalHealth, N.D.). Surgical gowns can be grouped into 4 classes based on their ability to resist liquid penetration. In order to ensure adequate protection against pathogen transfer, gowns are selected based on the expected degree of contamination, procedure duration as well as the clinician's role during the surgery (CardinalHealth, N.D.).

Despite the above measures, it has been estimated that surgical staff donning appropriate surgical attire still shed approximately 10 000 squamous epithelial cells per person per minute, with 10% of these cells being potential carriers of pathogenic micro-organisms (Al-Waked, 2010). The movement of each person can additionally cause re-suspension of settled microbial particles (Adams et al., 2015) which further contributes to the bioaerosol load.

Though optimizing the number of surgical personnel will certainly reduce the bioaerosol load, air filtration and distribution systems are necessary to reduce unavoidable contamination.

2.4.3.2 Air systems

As early as 1946 it was demonstrated by Bourdillon and Colebrook, as cited by Blowers and Crew (1960) that through the control of air movement, airborne contamination of the operating room can be minimized. Since then, various regulatory authorities, including but not limited to NIOSH (National Institute of Occupational safety and Health), ASHRAE (American Society of Heating, Refrigeration and Air-conditioning Engineers), AIA (American Institute of

Architects) and the CDC (Centers for Disease Control and Prevention), have developed standards for ventilation systems in surgical theatres (Maheshwari, 2012).

The concentration and distribution of air pollutants in the operating room is directly influenced by the quality of ventilation (Maheshwari, 2012). Not only does a well installed ventilation system dilute particulate matter, it additionally provides an infiltration barrier through room pressurization, alters air distribution patterns through directional airflow and furthermore removes contaminants through filtration (Maheshwari, 2012).

The CDC currently recommends that the operating room maintain a positive pressure with respect to surrounding corridors and adjacent areas (Owens and Stoessel, 2008, Singhal, 2018). Pressure differentials are usually maintained at 2.5Kpa (Maheshwari, 2012) between these areas which prevents air from moving from relatively less clean, to cleaner areas (Mangram et al., 1999, Chauveaux, 2015).

Recommendations with regards to the number of air changes per hour vary between advisory bodies. The CDC for example currently recommends a minimum of 15 air changes per hour of which at least 20% must be fresh air, with all air (whether recirculated or fresh) passing through appropriate filters (Owens and Stoessel, 2008, Mangram et al., 1999, Singhal, 2018). The WHO advises 20 air changes, whilst the AIA recommends 20-25 air changes per hour (Maheshwari, 2012). As air flow increases, so too does the dilutional effect thereof, this however needs to be balanced with the air velocity which can potentially increase air turbulence and thus increase re-suspension of settled particles (Maheshwari, 2012).

Ventilation systems can be divided into two main configurations, the conventional system in which air moves in a turbulent manner, or a unidirectional system namely laminar flow, in which, as the name implies, air moves in a single direction either horizontally or vertically (WHO, 2016). Each configuration has its own proposed benefits: the turbulence created by conventional systems quickly homogenizes the air, leading to rapid dilution of particulate matter (WHO, 2016); the parallel airflow created by laminar flow systems on the other hand, theoretically minimizes turbulence, thereby creating stabilized flow over the surgical area (WHO, 2016) while also minimizing suspension of settled particles. For this reason laminar flow systems are often perceived to provide an ultra-clean environment and are thus found in

orthopaedic suites. Following an extensive literature review, the World Health Organization (2016) found that laminar airflow provided no benefit and non-significant beneficial effect for total hip and knee arthroplasty respectively, when compared to conventional ventilation systems. With no randomized control studies being available, these conclusions were based on data obtained from national databases (i.e. between hospitals and not within). This data is therefore considered to be of low quality and consequently the World Health Organization made a conditional recommendation that "laminar airflow ventilation systems should not be used to reduce the risk of SSI for patients undergoing total arthroplasty surgery" (WHO, 2016).

Another important consideration is the source of air. Normal air that one breathes in is comprised of not only gases, but numerous particulates of which one is bacteria. Considering the ubiquitous nature of bacteria, it is equally important that air entering the OR does not serve as an additional source of pathogenic organisms (Melhado et al., 2006). For this reason, the air needs to pass through a high efficiency filter prior to entering the operating room (OR) to remove bacteria. Bacteria generally vary in size from 0.2 to 5μm (Chauveaux, 2015). These organisms can then adhere to larger particles (such as dust or squamous epithelial cells) and form aggregates known as colony forming units (CFUs) (Chauveaux, 2015). A positive association has been shown between bacterial colony counts and particles in excess of 5μm in size. It was concluded that all particles in excess of 5μm can be considered potentially infective (Chauveaux, 2015). Although in use for nearly 80 years, high efficiency particulate air (HEPA) filters are still considered to be the "best in class method for removing infectious particles from air" (Fernstrom and Goldblatt, 2013). These filters are able to remove particles larger than 0.3μm with an efficiency of 99.97% (Mangram et al., 1999, WHO, 2016, Shaw et al., 2018). Because of this, the installation and maintenance of these filtration systems in human hospital theatres is thus a legal requirement in many countries (WHO, 2016).

Ultimately the above-mentioned air control systems aim to reduce the airborne particulate count. The efficacy of these systems can be quantified in terms of colony forming units per meter cubed. Bacterial counts in conventionally ventilated theatres are currently limited to 35 CFU/m^3 in the UK, 25 CFU/m^3 in Switzerland and 5 CFU/m^3 in France (Chauveaux, 2015). Though international consensus with regards to acceptable limits have not been reached, the prevailing mind-set is clear, airborne bacterial contamination poses a significant surgical site infection risk and measures should thus be in place which limit this risk. Though the risk may be recognized in the veterinary industry, in the author's observations, only a small number of

practices, other than a specialized few, have the resources necessary to control the airborne bacterial load in their surgical theatres.

Despite all the above measures, infection prevention cannot be guaranteed. Thus in cases where SSI's are more probable as a result of the patient's ASA status, the type of procedure or where the consequences of infection would be devastating, prophylactic antimicrobial therapy is employed to mitigate the risk.

2.5 Surgical antimicrobial prophylaxis

2.5.1 Timing of administration

As early as 1961, experimental studies conducted by Burke demonstrated that "antibiotics cause maximum suppression of infection if given before bacteria gain access to tissue" (Gawande et al., 2009, Burke, 1961). Since its publication, placebo controlled studies have validated this claim by demonstrating reduced surgical infection rates following prophylactic antimicrobial administration (Gawande et al., 2009). To this effect, a randomized, controlled, double blind trial which compared the effect of administering cefazolin, an antimicrobial, at two different timings during caesarean section was conducted (Jyothirmayi et al., 2017). A total of 1106 patients formed part of the trial with 553 mothers receiving 1g of cefazolin IV preoperatively, whilst the remaining 543 were dosed intra-operatively after cord clamping. It was found that not only did the pre-operative dose group of patients experience a significantly lower incidence of post-operative febrile events, but that surgical site infections were additionally substantially lower in this group as well (0.4% vs. 4.6% with a p value of <0.001) (Jyothirmayi et al., 2017). This lead the authors to conclude that the administration of intravenous antibiotics to mothers prior to skin incision for caesarean section lead to an overall decrease in "postoperative infectious morbidity in the mother" (Jyothirmayi et al., 2017).

Prophylactic antimicrobial therapy is employed with the goal of reducing the risk of surgical site infection, while at the same time minimizing the impact thereof on the patient's microbiota (Jocum, 2018). When employed empirically, it furthermore reduces overall antibiotic use, thus helping to curb the rapidly escalating antimicrobial resistance threat (Weese, 2008, Weese et al., 2015, Rantala et al., 2004, Morley et al., 2005). Prophylactic antimicrobial therapy however needs to be employed in the correct manner in order to achieve these goals.

Timing of administration is vital to the success of prophylactic therapy. Bacterial contamination will only progress to infection if the host defence mechanisms are overwhelmed (Verwilghen and Singh, 2015). By ensuring adequate plasma and tissue antimicrobial concentrations, bacterial multiplication can be curbed to the extent that the host defence mechanisms can prevent the progression thereof to infection (WHO, 2016, Verwilghen and Singh, 2015). In order to achieve adequate plasma concentrations at first challenge (i.e. upon first incision), the World Health Organization strongly recommends that intravenous antimicrobial administration should take place no longer than 2 hours prior to the start of the procedure (WHO, 2016). The exact pre-surgical dosing interval should however be optimized to suit the antimicrobial agent's half-life (WHO, 2016). The commonly used agent cefazolin, for example, due to its relatively short half-life of 90 minutes (Kusaba, 2009), in combination with the fact that it is a time dependant antimicrobial and antibacterial activity requires that the drug concentration remain above the minimum inhibitory concentration for a specific duration (Kusaba, 2009), be given no longer than 1 hour prior to first incision (WHO, 2016). The rapid decline in serum concentration of this agent after intravenous administration is depicted in figure 2.2 below

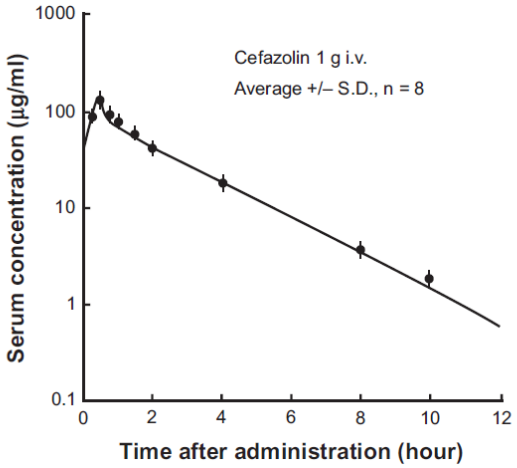

Figure 2-2 Serum concentration of cefazolin after 1g of intravenous administration in patients with normal renal function

Reproduced from Kusaba (2009)

Though complete consensus has not been reached among all advisory bodies, a vast number thereof, including but not limited to the USA Institute of Health Improvement, UK High impact

intervention care bundle, the Royal College of Physicians of Ireland, Society of Healthcare Epidemiology of America and the World Small Animal Veterinary Association, agree with the administration of prophylactic antimicrobials within the 60 minute window (WHO, 2016, Spohrc et al., 2012, Trepanier, 2013).

2.5.2 Repeat dosing

In 2002, Zelenitsky et al. (2002) demonstrated the correlation between decreased plasma antimicrobial concentrations upon surgical incision closure and increased post-operative surgical site infections. This was done by conducting a pharmacodynamic analysis on data obtained from a previously conducted prospective, randomized, double blind clinical study which compared two regimens of gentamicin for prophylaxis for colorectal surgery. In the original study, 68 patients received either a single high dose of gentamicin (4.5mg/kg) preoperatively, whilst 66 received multiple standard doses of gentamicin (1.5mg/kg) preoperatively as well as at 8, 16 and 24 hours post operatively. In both groups 500mg of metronidazole was also administered. The development of surgical site infections within 30 days was the primary outcome. Utilizing blood samples drawn 30 minutes after the administration of the pre-operative dose as well as upon recovery, pharmacokinetic curves were created and used to predict the concentration at the time of incision, as well as at time of closure. It was subsequently determined that "the gentamicin concentration at the time of surgical closure was one of the strongest independent risk factors for infection" (Zelenitsky et al., 2002) with 1.6mg/litre being the critical closure concentration for surgical prophylaxis and concentrations below 0.5mg/litre being associated with infection rates of 80% (Zelenitsky et al., 2002).

It is thus important to maintain adequate plasma concentrations for the duration of the challenge (WHO, 2016, Bratzler et al., 2013). It has been recommended by the American Society of Health System Pharmacists that the antimicrobial should be re-administered should the procedure duration extend beyond two drug half-lives, or if there is excessive blood loss (Bratzler et al., 2013). A study conducted by Scher (1997) demonstrated the importance of this practice. In their study, in which 801 patients underwent elective clean contaminated surgery, it was found that in surgeries exceeding 3 hours, the group that had received 1g of cefazolin

preoperatively and another dose 3 hours later had a significantly decreased wound infection rate when compared to those that had only received 1g of cefazolin preoperatively.

This line of thinking in which antimicrobials are re-administered after every two half-lives as a way of maintaining therapeutic concentrations seems to have extrapolated to the veterinary field – in a veterinary article written by Verwilghen and Singh (2015) the same conclusion was reached.

2.5.3 Discontinuation of therapy

Best practice guidelines, including those recommended by the American Society of Health System Pharmacists (ASHP), recommend the discontinuation of surgical antimicrobial prophylaxis within 24 hours after wound closure (Bratzler et al., 2013). A recent extensive literature review conducted by the WHO (2016) further refined these recommendations. After analysing data from 69 randomized controlled trials, covering over 21 000 patients it was concluded that prolonging surgical antimicrobial prophylaxis (SAP) beyond doses given intra-operatively, did not further decrease the surgical site infection rate (WHO, 2016). Examples of some of the studies analysed by the WHO are available in Table 2-3.

It can thus be concluded that, not only is there no evidence of a further reduction in surgical site infection with protracted surgical antimicrobial prophylaxis (SAPs), but this practice contributes to increased antimicrobial resistance (Anderson et al., 2014, Antonioli et al., 2018). Though recommended before the release of the WHO review, recommendations made at the 2013 World Small Animal Veterinary Association World Congress, which states that antimicrobials should be discontinued after placement of the last skin suture, are in line with the above findings (Trepanier, 2013).

Table 2-3 Studies evaluating surgical site infection rates in patients who only received prophylactic antimicrobial therapy, in comparison to patients that received prolonged antimicrobial therapies post operatively

Study type	No. of patients	Surgery Type	Protocol	Infection type	SSI infection rates		Author Conclusions	Reference
					Prophylactic & intraoperative only	Prophylactic, Intraoperative & Extended		
Randomized study	370	Elective colon cancer surgery	Group A: Single 1g dose of Flomoxef immediately prior to surgery Group B: 1g Flomoxef immediately prior to surgery + 1g Flomoxef twice daily until post- operative day 3	Incisional	8.40%	7.20%	Single preoperative dose is sufficient as perioperative infection prophylaxis	(Suzuki et al., 2011)
				Organ Space	0.60%	1.10%		
				Remote	4.50%	3.30%		
Randomized study	257	Esophagectomy (due to cancer) with three field lymph node dissection	Short course administration group: Administration 30min prior to skin incision and repeated every 3 hours during procedure Prolonged administration group: Same as short course administration group, but continued antimicrobial therapy	SSI	26.50%	24.00%	Short course antimicrobial administration was sufficient	(Fujita and Daiko, 2015)
				Anastomotic leakage	11.70%	15.50%		
				Incisional infections	14.80%	8.50%		
				Remote infections	11.70%	11.60%		
Prospective, open label phase 3, randomised controlled non-inferiority trial	355	Gastrectomy	Group 1: cefazolin 1g before surgery and every 3h intraoperatively Group 2: Same as group one with the addition of 1g of cefazolin after closure and twice daily for 2 postoperative days	SSI	5.00%	9.00%	Statistically significant non-inferiority. Elimination of antimicrobial prophylaxis did not increase SSI incidence	(Imamura et al., 2012)

Study type	No. of patients	Surgery Type	Protocol	Infection type	SSI infection rates		Author Conclusions	Reference
					Prophylactic & intraoperative only	Prophylactic, Intraoperative & Extended		
Randomized controlled non-inferiority trial	176	Caesarean section in a low resource setting	Intervention group: single dose of ampicillin (1000mg) and metronidazole (500mg) IV 20min preoperatively Control group: 500mg metronidazole and 500mg ampicillin IV at 8 and 16hours post operatively followed by 500mg amoxicillin and 400mg metronidazole per os 3 times a day for 2-5 days	SSI	6.70%	10.30%	Single prophylactic dose is equally effective in preventing wound infections	(Westen et al., 2015)
Prospective randomized	548	Gynaecological procedures (Hysterectomy or ovarian cystectomy for non-malignant disease	Single dose group: 1g of cefazolin IV before surgery Multiple dose group: 1g cefazolin IV before surgery and three more doses every 6 hours after surgery	SSI	0.37%	0.37%	The use of single dose prophylaxis was as effective as four doses of cefazolin. Shortening duration of administration will reduce costs and microorganism resistance	(Su et al., 2005)

2.5.4 Antimicrobial selection

The selection of an appropriate antimicrobial is an important factor in prophylactic efficacy (Owens and Stoessel, 2008, Nelson, 2011). Antibiotic selection is based on the susceptibility of the most likely contaminating organisms (Owens and Stoessel, 2008, Jocum, 2018, Bratzler et al., 2005). For ease of use, guidelines which categorize anticipated pathogens in respect to organ system or procedure type are available in both the human medical and veterinary fields (Verwilghen and Singh, 2015, Enzler et al., 2011). Though overlap may exist, it is best to follow species specific guidelines. In the veterinary field, an article written by Verwilghen and Singh (2015) relates procedure type, anticipated pathogens and consequent recommended antimicrobials. The table presented in their article has been combined with recommendations made by Fossum (2007) in table 2-4 below

Table 2-4 Antimicrobials recommended for surgical procedures in small animals based on anticipated pathogen(s)

(Fossum, 2007, Verwilghen and Singh, 2015)

Procedure	Anticipated Pathogen(s)	Recommended Antimicrobial
Abdominal surgery	*Staphylococcus* spp.	cefazolin
Cardiothoracic	*Staphylococcus* spp.	cefazolin
Gastrointestinal		
Oral	Gram-positive aerobes and anaerobes	ampicillin clindamycin
Oesophageal surgery	Anaerobes	clindamycin ticarcillin + clavulanic acid cefoxitin
Upper GIT	Gram-positive cocci (e.g. *Staphylococcus*, *Enterococcus*), Enteric Gram-negative bacilli (e.g. *Escherichia coli*, *Bacteroides*)	first generation cephalosporin's e.g. cefazolin
Lower GIT	Enterococci, Gram-negative bacilli, anaerobes	second generation cephalosporin's e.g. cefoxitin
Ruptured bowel	Gram-positive cocci, enteric Gram-negative bacilli, anaerobes,	ampicillin & fluoroquinolone
Hepatobiliary surgery	Gram-negative bacilli, Clostridia spp., anaerobes	cefoxitin
Orthopaedic & Neurologic		
Elective procedures, closed fractures, spinal decompression	*Staphylococcus* spp.	cefazolin
Open fractures	*Staphylococcus* spp., *Streptococcus* spp., anaerobes	cefazolin
Skin and reconstructive surgery	*Staphylococcus* spp. particularly *S. pseudintermedius*	cefazolin
Urogenital surgery	*Escherichia coli, Streptococcus* spp., anaerobes	cefazolin ampicillin
Note: If infection is present, culture and sensitivity should ideally be done first		

The above guidelines place emphasis on 'correct' prophylactic antimicrobial therapy, namely selecting the correct antimicrobial for the encountered pathogens, obtaining and maintaining

suitable plasma and tissue concentrations as well as discontinuing therapy after an appropriate duration. These measures are important because the use of antimicrobials in any form, whether for therapeutic or prophylactic purposes, increases the selection pressure on not only the target organisms, but importantly on the commensal microbiota as well (Weese et al., 2015). This in turn perpetuates the development of antimicrobial resistance, a problem which has become so immense in both the human and animal health sectors that in 2019 it was listed as among the top 10 global health threats by the World Health Organization (WHO, 2019b).

2.6 Antimicrobial use in veterinary medicine

Though guidelines, such as the antimicrobial consensus statement provided by the American College of Veterinary Internal Medicine (Morley et al., 2005), which recommend appropriate antimicrobial use practices in veterinary medicine, are available, these guidelines are not always followed on a clinical level.

In a 2016 surgical national antimicrobial prescribing survey conducted in Australia, it was determined that "procedural antimicrobial prophylaxis was prescribed, but not indicated in 10% of surgical procedures" (Ierano et al., 2017). Antimicrobial administration is furthermore often prolonged in an "attempt to reduce the incidence of SSI" (Antonioli et al., 2018, p.139), a practice which has not only been proven to be ineffective for this purpose, but furthermore contributes to the growing antimicrobial resistance threat (Antonioli et al., 2018).

The same concern has been noted for the antimicrobials used for therapeutic purposes. In a study conducted by Shea et al. (2011) which investigated the therapeutic antibiotic use patterns in dogs in a veterinary teaching hospital, it was found that only 17% of therapeutic antibiotic prescriptions were for confirmed bacterial infections, the remainder being prescribed for either suspect infections (45%) or cases in which there was no evidence of infection (38%) (Shea et al., 2011). In this particular study, amoxicillin-clavulanate, was the most commonly prescribed antibiotic forming 24% of total prescriptions (Shea et al., 2011). The broad spectrum nature of this drug makes it an inappropriate choice for use as a first line agent, unless selection thereof has been based on a culture and sensitivity (Shea et al., 2011).

In a survey, evaluating condition based prescribing of antimicrobials in a veterinary hospital conducted by Rantala et al. (2004), post-operative antibiotic use was quantified. It was

determined that post-operative antimicrobial therapy accounted for 12% of all antimicrobials administered. Beta-lactams and trimethoprim sulphonamides alone accounted for 87% of the antimicrobials used for this purpose. Ultimately, the authors concluded that "antimicrobial drugs were used excessively after surgical procedures" (Rantala et al., 2004, p.259).

Concern is also expressed with the volume of antimicrobials used in small animal medicine, even in countries regarded as exemplar such as Denmark. Data provided by VetStat, which is run by the Danish Veterinary and Food Administration agency of the Ministry of Environment and Food, allowed for evaluation of trends on a larger scale. Though the total consumption of antimicrobials used in dogs and cats has fallen by 10% since the first publication of antibiotic use guidelines for companion animal practice by the Danish Veterinary Association in 2012, there has been a sharp increase in amoxicillin clavulanate use in the same period, so much so, that by 2016 it made up 53% of dispensed preparations (Jessen et al., 2018). The figure depicting the Danish Veterinary Associations findings has been reproduced in Figure 2-3 below (Jessen et al., 2018)

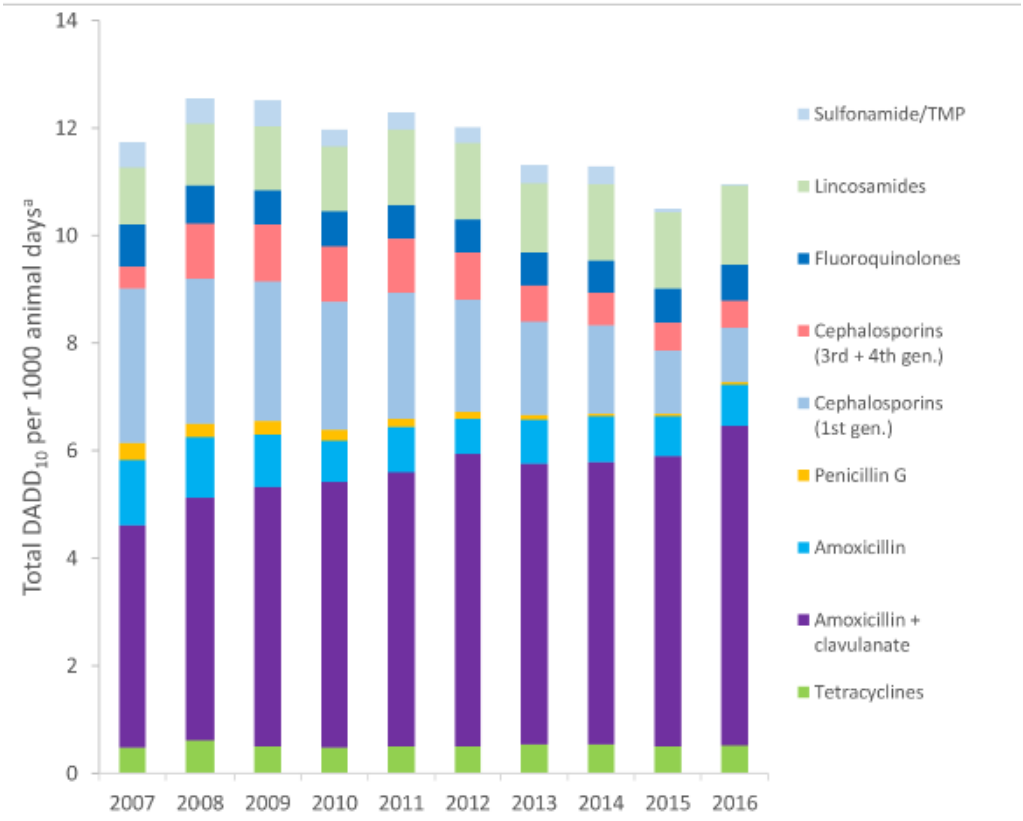

Figure 2-3 Developments in the use of systemic antibiotics for the treatment of companion animals, for the most commonly used antibiotic classes

Reproduced from (Jessen et al., 2018)

Note: With the exception of cefovecin which is a parenteral formulation, the above only takes into account orally administered medication. It was furthermore assumed that small animal population remained stable over this period.
DADD (Standardized daily dose) assumed a 10kg average body mass for all small animals. (Jessen et al., 2018).

Though the above studies did not focus solely on the treatment of surgical site infections, it is likely that amoxicillin/clavulanate was used for surgical prophylaxis, with the consequences of imprudent antimicrobial use remaining the same.

With the indiscriminate antibiotic use in human medicine known to contribute to growing antibiotic resistance (Shea et al., 2011), it is reasonable to assume that inappropriate antibiotic use in the veterinary field, as demonstrated above, will lead to the same net result.

2.7 Antimicrobial resistance

Antimicrobials are instrumental tools in the treatment of bacterial pathogens. Failure of the efficacy of these drugs not only poses a substantial economic burden, estimated to be in excess of twenty billion US dollars per annum in the United States alone (Munita and Arias, 2016), but it furthermore considerably increases patient morbidity and mortality. At present bacterial resistance is responsible for an estimated 700 000 deaths globally each year (Lerminiaux and Cameron, 2019).

2.7.1 Types of resistance

Bacterial resistance may be divided into two categories, the first of which would be constitutive resistance (Morley et al., 2005). These chromosomally encoded resistance mechanisms are generally non-specific but provide the organisms with an inherent structural or physiological adaptation which makes them inherently resistant to a specific subset of antimicrobials (Morley et al., 2005, Peterson and Kaur, 2018). The transport of aminoglycosides across the cytoplasmic membrane for example, is an active, oxygen dependent process. Due to their specific niche conditions, anaerobic bacteria therefore have an innate resistance to this antibiotic class (Munita and Arias, 2016, MacDougall, 2018). Acquired resistance on the other hand is considered an evolutionary adaptation which can arise either as a result of gene mutation or through the horizontal transfer of so-called 'resistance genes' (Morley et al., 2005, Munita and Arias, 2016). It is this second category of antimicrobial resistance, namely acquired resistance, on which emphasis is placed when referring to the development of antimicrobial resistance in a clinical setting (Munita and Arias, 2016). Only acquired antimicrobial resistance mechanisms will thus be discussed below.

2.7.2 Acquired resistance

Bacteria are organisms with enormous genetic plasticity (Munita and Arias, 2016), so much so, that genetic mutations are thought to occur at a rate of "1 mutation per million bases per cell division" (Morley et al., 2005, p.619). It is thus not the exposure to antimicrobials which cause organisms to actively mutate, but rather the vast genetic variation within a species which allows those organisms which, per chance, possess a favourable selective advantage, to prosper and replicate in the presence of the antimicrobial (Morley et al., 2005).

Genetic mutations confer antimicrobial resistance through four key mechanisms; modification of the drug target, decreased drug uptake, increased efflux thereof, or through the enzymatic destruction or alteration of an antimicrobial (Reygaert, 2018). Once these genetic mutations are present, the mutated bacteria have a distinct competitive advantage in the presence of the antimicrobial drug. Whether or not these genetically variant organisms persist in the absence of antimicrobial pressure however, depends on whether or not the mutation affected other fundamental homeostatic mechanisms (Morley et al., 2005, Munita and Arias, 2016).

Though genetic mutation plays a critical role, it is thought that horizontal gene transfer (HGT) is the main perpetuator of antimicrobial resistance (AMR) (Munita and Arias, 2016). Horizontal gene transfer is a collective term referring to the acquisition of foreign genetic material through bacteriophage mediated processes (transduction), incorporation of extracellular DNA (transformation), or most commonly, through conjugation whereby antimicrobial resistance genes (ARG) are transferred from one organism to another by means of independently replicating genetic elements known as plasmids (Morley et al., 2005, Lerminiaux and Cameron, 2019, Peterson and Kaur, 2018). Methicillin resistant *Staphylococcus aureus*, better known as MRSA, is a well-known example of an organism with acquired resistance genes. These organisms have acquired a non-native mecA gene which encodes a modified penicillin binding protein, namely PBP 2a. Penicillin binding proteins (PBP's) are involved in the transglycosylation and transpeptidation stages of peptidoglycan synthesis, a critical component of the bacterial cell wall (Fergestad et al., 2020, Sauvage et al., 2008). Under ordinary circumstances β lactam antimicrobials are able to bind to PBP's, forming covalent bonds which inactivate these enzymes and consequently inhibit the cross linking of glycan chains (transpeptidation) (Sauvage et al., 2008, Fisher and Mobashery, 2016). Unlike other PBP's which serve as an antimicrobial target, PBP2a has an inherently low affinity for β-lactam antibiotics. Bacterial cell wall synthesis can therefore continue in what would otherwise be inhibitory concentrations of these antibiotics (Verwilghen and Singh, 2015, Fergestad et al., 2020, Peacock and Paterson, 2015, Llarrull et al., 2009).

HGT ultimately allows for the rapid diversification of a species by allowing for the acquisition of ARGs that would normally not form part of the genomic sequence (Lerminiaux and Cameron, 2019). This diversification is however not limited to a bacterial strain as horizontal gene transfer allows these ARG's to jump between species (Morley et al., 2005, Lerminiaux and Cameron, 2019). *Staphylococcus pseudintermedius*, the veterinary equivalent of *S. aureus*,

displays this same PBP2a adaptation (Fang, 2015). Though there is no evidence of 'direct gene transfer between these species', Bannoehr et al. (2007) were able to show a high degree of homology (90-100%) between the mecA genes in these species. Because this gene is so mobile, various authors postulate that direct transfer is in fact possible between *S. aureus* and *S. pseudintermedius* (Wladyka et al., 2015).

Through horizontal gene transfer, methicillin resistance has become widely distributed in the *S. pseudintermedius* species (Fang, 2015, Bannoehr et al., 2007). Methicillin resistant *S. pseudintermedius* (MRSP) has consequently become a significant problem in the veterinary industry (Burgess, 2019, Verwilghen and Singh, 2015, Wladyka et al., 2015, Nelson, 2011). In a study conducted at the Ontario Veterinary College Health Sciences Centre, it was found that MRSP caused 64% of the cultured confirmed surgical site infections (Turk et al., 2015). Apart from their resistance to beta-lactam antibiotics, some MRSP isolates have been shown to be resistant to multiple drug classes, including tetracyclines, macrolides, lincosoamides, aminoglycosides, trimethoprim, chloramphenicol and fluoroquinolones (Fang, 2015), further limiting the availability of treatment options for infections caused by these organisms.

Of the organisms implicated in veterinary surgical site infections, *S. aureus* and *S. pseudintermedius* are not the only pathogens to display antimicrobial resistance. Coagulase-negative staphylococci, *Pseudomonas*, enterococci and extended spectrum B-lactamase producing *Enterobacteriaceae* have additionally displayed high levels of multidrug resistance (Verwilghen and Singh, 2015, Nelson, 2011).

Provided that the antimicrobial resistant gene does not compromise an organism's ability to compete within its own ecological niche, once present, it is unlikely that these antimicrobial resistance genes will disappear from an organism's genetic code (Morley et al., 2005). It is thus important to, through prudent antimicrobial use, minimize the selection pressure which favours the establishment of these ARGs. Decreased antimicrobial use furthermore allows non-resistant strains to continue proliferating, thereby diluting the strains that carry resistance determinants.

2.8 Prudent antimicrobial use

2.8.1 Global action plan

According to the World health organization, the antimicrobial resistance threat has escalated to such an extent that it is considered “a crisis that must be managed with utmost urgency” (WHO, 2015). Because of this, the WHO in conjunction with the World Organization for Animal Health (OIE) and the Food and Agricultural Organization of the United Nations (FAO) published a “Global Action Plan” on microbial resistance in 2015. This action plan, which by July 2018 was being implemented to varying degrees by 154 member countries, highlights five key objectives which centre on 1) improving awareness, 2) optimizing antimicrobial use, 3) further reducing the need thereof through the reduction of infection, 4) improving surveillance strategies and 5) encouraging sustainable investment aimed at countering antimicrobial resistance (WHO, 2015).

2.8.2 One health approach

In order for these strategies to be effective, a One Health approach, which applies collaborative input from all relevant stakeholders in the medical, veterinary and environmental sectors, at a local, national and global scale must be employed (FDA, 2018). The OIE, along with various other regulatory bodies, including but not limited to the AVMA (American Veterinary medical Association), the FDA, the IACG (Interagency coordination group on antimicrobial resistance) and the ACVIM (American College of Veterinary Internal Medicine) published recommendations regarding the responsible and prudent use of antimicrobials specifically for the veterinary industry which includes revised use guidelines for surgical antimicrobials (OIE, 2019).

2.8.3 Limiting unnecessary use

Because the use of any antimicrobials, whether it be for therapeutic or prophylactic purposes, places a selection pressure on all exposed bacteria (Weese et al., 2015), disease prevention is a principle that is emphasized in many of these guidelines (Weese et al., 2015, FDA, 2018). As previously discussed, the environmental conditions in an operating room play a deciding role in the degree of bacterial contamination of the surgical site and its consequent progression to infection, therefore, if implemented correctly, environmental management alone can reduce the

infection risk to such an extent that prophylactic antimicrobial therapy may not be required for certain procedures.

It is currently recommended that short (<90minute), clean, non-orthopaedic procedures carried out on veterinary patients classified as ASA 1 or 2 (i.e. low risk) do not require antimicrobial prophylaxis (Nelson, 2011, Griffin et al., 2016, Spohrc et al., 2012). This practice is in line with the American College of Veterinary Internal Medicine's recommendation that "it is not necessary to use antimicrobial drugs in all surgical cases to prevent infection" (Morley et al., 2005, p.626). These recommendations echo the practices employed in the human medical field.

According to guidelines developed jointly by the American Society of Health-System Pharmacists (ASHP), the Infectious Diseases Society of America (IDSA), the Surgical Infection Society (SIS) and the Society for Healthcare Epidemiology of America (SHEA) "antimicrobial prophylaxis may be beneficial in surgical procedures associated with a high rate of infection (i.e., clean-contaminated or contaminated procedures) and in certain clean procedures where there are severe consequences of infection (e.g., prosthetic implants), even if infection is unlikely" (Bratzler et al., 2013, p. 81). Both the WHO and CDC similarly recommend that antimicrobial prophylaxis should only be administered "when indicated" depending on the type of operation and based on clinical practice guidelines (Berríos-Torres et al., 2017).

Depending on local factors, including local antimicrobial resistance patterns, the availability of antibiotics and the patient population, some guidelines may vary slightly in their recommendations (Jocum, 2018). Procedures for which it is appropriate to omit human surgical antimicrobial prophylaxis in a South African setting are available in Table 2-5 below.

Table 2-5 Human surgical procedures for which antimicrobial prophylaxis is not recommended in a South African setting

Partly reproduced from Jocum (2018)

	Antimicrobial prophylaxis not recommended
Facial surgery	Clean facial surgery
Ear nose and throat (benign)	Ear surgery (clean/clean contaminated)
	Endoscopic sinus surgery
	Tonsillectomy and adenoidectomy
Head and neck	Clean benign procedures
Hepatobiliary	Laparoscopic cholecystectomy (may consider if complicated)
Abdomen	Hernia repair
	Diagnostic endoscopy
Obstetrics and gynaecology	Evacuation of incomplete miscarriage
Urology	Transurethral bladder tumour resection
	Circumcision
Limb surgery	Orthopaedic surgery without implant

In cases where prophylactic antimicrobial therapy is deemed necessary, the chosen agent should cover the expected pathogens and care should be taken to administer it in the appropriate dose and at the correct time intervals (Jocum, 2018) as previously discussed.

However, despite all possible preventative measures, surgical site infections will still have an occurrence rate of between 2.5 and 28% depending on the type of veterinary surgical procedure (Spohrc et al., 2012, Turk et al., 2015, Vasseur et al., 1988, Shales, 2012), and thus, the use of antimicrobials cannot be altogether avoided.

At present, no guideline is available for use in animals. For humans, the National Institute for Health Care and Excellence (NICE) recommends that should surgical site infection be suspected, the patient should immediately be placed on antibiotics that will cover the likely inciting organisms. They further recommend that sensitivity testing should take place and that local antibiotic resistance patterns be taken into account when choosing an antibiotic (NICE, 2019). While designed for human use, the application thereof can be extended to the veterinary

industry as the principles, which promote prudent antimicrobial use, are in line with veterinary antimicrobial stewardship recommendations (FDA, 2018).

Though the above only advises on the antimicrobial practices of surgical cases, a well applied antimicrobial stewardship program has a much wider scope in a veterinary practice setting. By advocating for judicious antimicrobial use with frequent reflection on use practices, promoting education, and endorsing infection prevention (FDA, 2018), these guidelines aim to provide "practical measures and recommendations intended to improve animal health and animal welfare while preventing or reducing the selection, emergence and spread of antimicrobial-resistant bacteria in animals and humans" (OIE, 2019).

2.9 Expected state of veterinary theatres

The cost of veterinary care seems to be a topic frequently up for discussion. Reporting on the findings of a survey conducted by the British Veterinary Association, the Telegraph, a UK based news agency, stated that "85% of vets said they or a member of their team, had felt threatened by a client's language or behaviour" (Sawer, 2017). Aggression over cost of care was additionally noted to be on the rise (Sawer, 2017). It therefore comes as no surprise that many veterinarians try to minimize costs however possible. Unfortunately it seems highly possible that cost cutting takes place in veterinary theatres. Ideally a veterinary theatre should provide a sterile environment in which to perform surgery. This 'ideal' environment, which has been discussed extensively above, cannot however be achieved without a fairly substantial initial financial investment followed by on-going expenditure to maintain the equipment and facilities.

It is expected that few, non-specialist veterinary practices, have adequate ventilation systems, that full surgical attire is not strictly adhered to, that surgical sets are not always adequately sterilized between patients and that post-surgical antimicrobial therapy is utilized as a means of compensating for these failures. These 'non-ideal' conditions are to an extent facilitated by regulatory guidelines which are left open to interpretation.

2.9.1 Ventilation systems

The South African Veterinary Council for example simply states that it requires 'adequate ventilation' in operating rooms (SAVC, 2016). Nowhere does it provide further explanation as

to what is considered ‘adequate’. In many instances natural ventilation is therefore the sole means of air control in veterinary practices. Though the World Health Organisation does not currently make recommendations with regards to naturally ventilated theatres as a feasible alternative to artificially ventilated operating rooms due to a lack of evidence (WHO, 2016), they do emphasise the importance of a proper ventilation rate and “adequate maintenance of the components of the installed ventilation system” (WHO, 2016). This statement in itself assumes the availability of some form of ventilation system for human surgery as a must, a component which is often missing in the ‘average’ veterinary practice. Due to the importance of the bioaerosol composition, lack of an adequate ventilation system could potentially lead to an increased surgical site infection rate. This was demonstrated in a 2012 study conducted by Song et al. (2012) in which a statistically significant increase in surgical site infections in humans was demonstrated when procedures that had been performed in non-artificially ventilated theatres were compared to theatres supplied with HEPA filtration (Song et al., 2012).

2.9.2 Surgical attire

The SAVC furthermore requires that “aseptic conditions be maintained in the operating room” (SAVC, 2016). Though it can be assumed that this requirement encompasses surgical attire, it is not specified anywhere. Donning complete surgical attire (including mask, sterile gloves, head cover and disposable surgical gown) however will cost approximately R60.00 per surgical staff member per patient (as calculated using surgical attire products listed by Azulwear in 2019). Of this expense, the surgical gown accounts for approximately 90%. Some veterinary facilities, particularly high volume – low cost spay-neuter facilities may not have the funds for this. The Association of Shelter Veterinarians’ 2016 Veterinary Medical Care Guidelines for Spay-Neuter programs (Griffin et al., 2016) for example, states that the use of a sterile surgical gown is “left up to the discretion of the surgeon” and that examination gloves are acceptable for cat and puppy castrations (Griffin et al., 2016).

2.9.3 Patient preparation

Though stated in the SAVC guidelines that patients must be prepped in a separate room and that there may be no thoroughfare through the operating room (SAVC, 2016), the daily application of these aspects are difficult to evaluate on a once off visit by an inspection committee and thus, it is assumed that in at least some cases, these regulations are not being adhered to with some patients being prepared in the operating room.

2.9.4 Antimicrobial use

Minimizing the incidence of surgical site infections and consequent antibiotic use requires a multifaceted approach. Failing to adhere to the above mentioned practices would contribute to an increased bacterial load in operating rooms. This, may in turn, lead to increased post-operative antimicrobial use which could otherwise have been avoided. By simply altering the management practices in veterinary theatres it is thought that the overall antimicrobial use can be decreased.

For this pilot study the aim was to evaluate the airborne bacterial load encountered in veterinary theatres during routine sterilization procedures of canines and felines and correlate these findings with the management practices employed by the facility. The collected isolates were furthermore tested for their antibiotic susceptibility to gain perspective on the resistance phenotypes of these organisms. From the results of the study, we expect to be able to conduct a more extensive study where actual infection rates are also taken into account.

3. METHODS AND MATERIALS

3.1 Sample size

For this study, four veterinary facilities were chosen for evaluation. Included were three facilities without ventilation which were considered to be high, intermediate and low throughput facilities. Comparison was made with the experimental animal theatre of the Faculty of Veterinary Science, University of Pretoria, as the facility was equipped with a HEPA ventilation system.

3.2 Selection of facilities

Four veterinary facilities with differing surgical caseloads were selected to form part of the study. Three were first opinion small animal veterinary practices without ventilation systems whilst the fourth facility, namely the Biomedical Research Centre (Onderstepoort, University of Pretoria) being equipped with a HEPA filtration system was included in this study as Facility D to serve as a 'control'. Facility conditions are discussed in more detail below.

3.2.1 Facility A

Facility A was a relatively high throughput practice, performing approximately 10 or more canine and feline ovariohysterectomies and orchidectomies on a daily basis. Though it is used solely as a surgical suite, the operating room was not equipped with a ventilation or air filtration system in any form. Procedures were however put in place to minimize contamination of the room. Surgical preparation of the patient was performed prior to transporting the patient to the operating room. Once inside, the single access door was closed and remained so for the duration of the procedure. It was only opened on the rare occasion that a staff member had a brief query. Ordinarily the surgeon was the sole member of staff within the operating room (OR), for data collection purposes however, a single additional person remained in the room to allow for handling of plates without compromising the sterility of the surgeon. The surgeon donned a mask, scrub cap and non-surgical, but clean gloves for each procedure. The patient was additionally covered with sterile drapes.

3.2.2 Facility B

Facility B was a medium throughput practice, performing approximately 4-5 procedures a day. These procedures were not necessarily limited to ovariohysterectomies and orchidectomies, though only procedures classified as such were sampled. The theatre was cooled by an air conditioner that only cooled internal air (i.e. no fresh air circulation) and was equipped with three doors, one of which lead directly to the outside environment. The majority of patient preparation was performed prior to moving the animal to the theatre; once there, sterile drapes were placed. Theatre personnel remained between 3 and 6 individuals. Sterile gloves were utilized by staff members in direct contact with the incision site, whilst other personnel utilized protective equipment either inconsistently (masks) or not at all (scrub caps and surgical gowns). Movement was generally not restricted and consequently an intermediate to high throughput throughout procedures was observed – the majority of personnel movement was through the two inside doors, which either remained open or were opened frequently, with occasional but rare movement through the outside door in a limited number of procedures.

3.2.3 Facility C

Facility C was a relatively low throughput practice, averaging approximately 2 surgical procedures a day. The theatre was equipped with an air conditioner as for Facility B which was utilized in all procedures. On 11 of the 13 occasions, patient preparation, including shaving and scrubbing was performed in the theatre itself; thereafter patients were covered with clean but non sterile drapes. Access to the surgical suite was limited with all 3 access doors remaining closed for the duration of the procedure in the majority of cases. The 2-4 staff members who were present remained fairly stationary and consequently minimal movement was observed within the operating room.

3.2.4 Facility D

Facility D consisted of 4 theatres, all of which were in use and thus sampled simultaneously. Procedures were such in nature that they would continue for an extended period of time, thus in order to more closely replicate other sampling conditions, settle plates were placed for the first 20 minutes (i.e. the average sampling time at the other practices) only. Two settle plates were placed per theatre – one next to the patient, just off of the sterile drapes, whilst the other was placed on the anaesthetic machine. Theatres 1 and 2 were equipped with HEPA filtration systems which had been turned on approximately 3 hours prior to the start of the procedures. Each theatre had one door leading out of the theatre and an additional door that allowed for movement between the two theatres. Theatres 3 and 4 were set up in a similar manner to

theatres 1 and 2, however without the addition of HEPA filtration. Because only a limited amount of data was collected at Facility D, in order to more accurately evaluate trends, theatres 1 and 2, which are essentially identical, were treated as a single unit – namely D1. The same applies to theatres 3 and 4 which together formed D2.

The surgical attire donned by staff members were identical between facilities D1 and D2, with all staff members utilizing masks, surgical caps, full scrub suits and sterile gowns. Clean but non-sterile gloves were additionally used. Because the procedures being performed at Facility D were educational in nature (i.e. endoscopic training workshops) significant movement was observed between all theatres. Personnel numbers varied continuously throughout the time of sampling but generally between 3 and 7 people were present per theatre.

3.2.5 Overall facility conditions

The above mentioned theatre conditions are summarized in table 3-1 below

Table 3-1 Summary of facility conditions

	Facility A	Facility B	Facility C	Facility D	
				D1	D2
General Facility Information					
Facility Through-put	High throughput 10 or more sterilizations on a daily basis	Medium throughput ±4-5 procedures a day. Procedures not necessarily limited to sterilizations	Low throughput practice, approximately 2 surgical procedures a day	Facility only utilized approximately once a month	Facility only utilized approximately once a month
Theatre cleaning	Cleaned upon completion of procedures daily with tables being cleaned if wet/dirty. F10 in addition to a sodium hypochlorite (bleach) product was used.	Floor and tables cleaned twice daily with F10. Tables cleaned between procedures	Cleaned at the end of each day with a high foaming chlorinated detergent (SS112) . Between procedures the table was cleaned with a chlorhexidine based product.	F10 was used to clean the walls, floors and tables. An F10 fogger which aerosolized the disinfectant was additionally used.	The theatres were cleaned with F10. This included cleaning walls, floors and tables. An F10 fogger which aerosolized the disinfectant was additionally used.
Procedure Protocols					
Patient					
Preparation	Pre surgical scrub performed in a separate room	Pre surgical scrub performed in a separate room	Patient preparation performed in the theatre for the majority of procedures	Pre surgical scrub performed in a separate room	Pre surgical scrub performed in a separate room
Draping	Patient partially draped with sterile drapes	Sterile drapes were utilized to partially drape the patient in just over half of procedures. No drapes were utilized for the remaining procedures	Clean but non-sterile drapes used to partially cover patient	Patient draped fully with sterile drapes	Patient draped fully with sterile drapes
Anti-microbial use	Duplocillin IM, upon completion of procedure	Duplocillin IM during preparation	No antimicrobials given	Not applicable	Not applicable
Surgical Personnel/ Attire					
Number of surgical personnel present	Never more than 2	Varied between 3 and 6	Varied between 2 and 4	Varied, but generally 4 surgical personnel were present for the majority of the time	Varied between 3-7
Gloves	Non-surgical gloves utilized	Surgical gloves utilized	No surgical gloves utilized	Non-surgical gloves	Non-surgical gloves

	Facility A	Facility B	Facility C	Facility D	
				D1	D2
Face mask	Only worn by the surgeon	Varied greatly - from all staff members wearing masks to no members wearing masks, with variations in between	Masks never utilized	All personnel wore masks	Majority of personnel wore masks
Scrub Cap	Surgeon only	No members of staff	No member of staff	All personnel	All personnel
Surgical gown	Not utilized	Not utilized	Not utilized	All personnel	All personnel
Air Conditions					
Doors/ Entrances	One. Remained closed for the majority of the time (including between procedures)	3 doors, one of which lead directly outside. At least one inside door was open in all but one procedure. Two open inside doors were more common with all 3 being open during 2 procedures.	3 inside doors lead into the theatre. Doors were kept closed for the majority of procedures, with one door being left open on occasion.	2 Inside doors each (one of which as an inter-leading door between the two theatres. Both doors remained open	2 Inside doors each (one of which as an inter-leading door between the two theatres. Both doors remained open
Movement	Minimal movement	Intermediate to high movement	Minimal movement	High throughput with a lot of movement	High throughput with a lot of movement
HVAC systems	None	Air conditioner which was on for 16% of procedures	Air conditioner which was on for all procedures	HEPA filtered	No filtration unit

Note: The above results are indicative of the average conditions per practice (i.e. in the majority of sampling session). Conditions per individual procedure may vary

Key: IM-Intramuscular, HEPA – High efficiency particulate air, HVAC – Heating ventilation and cooling

3.3 Selection of cases

In order to quantify the bacterial load, settle plates were placed during canine ovariohysterectomies and orchidectomies as well during feline ovariohysterectomies. Feline orchidectomies were not included due to the minimal time it takes to perform this procedure. No surgical procedures were booked specifically for the purpose of this study, only previously arranged procedures, which would commence regardless of sample collection, were used.

3.4 Preparation of plates

The blood agar was prepared specifically for this study by Potchefstroom Veterinary Laboratory in compliance with their Standard Operating Procedure document VDS-GP-03/BA (Mhlongo, 2018c). The media was poured into sterile plates under a laminar flow cabinet. Once prepared, quality checks were performed to ensure both the sterility of the media as well as its ability to support growth and yield known characteristics (e.g. haemolysis or pigment) of positive/negative ATCC control strains. Prepared plates were stored, with the lid facing down, at 2-8 degrees Celsius until used.

3.5 Sample Collection

Thirty minutes prior to the commencement of sampling, the required number of plates were removed from the refrigerator to allow them to reach room temperature. Each plate was given a unique identification code which corresponded to a data sheet on which the conditions of the procedure were recorded.

One plate was utilized for each procedure. The settle plate was placed at the same height as the patient and as close as possible to the incision site without affecting the sterile field – a maximum of 1 meter from the incision site was allowed. To avoid falsely elevated plate counts, the plates were placed on the lateral aspect of the patient to ensure that patients were not breathing directly onto the plate.

The plates were opened upon first incision and closed upon placement of the last suture. Following collection, the plates were placed with the lid facing down and maintained at room temperature. The samples reached the laboratory for further processing within 4 hours of collection.

Only a single opportunity to collect samples at the Biological Research Centre (Facility D) presented itself. As a way of maximizing data collection in each of the four theatres, a settle plate was placed on the anaesthetic machine in addition to the one placed on the surgical table. Due to the extended nature of the training procedures, the plates were placed upon first incision and closed after 20 minutes so as to simulate the average duration of a surgical sterilization. To ensure the viability of the organisms, initial incubation and colony counts were performed on

site by the DVTD bacteriology laboratory which forms part of the Department of Veterinary Tropical Diseases at Onderstepoort. Subcultures were made so that colonies could be transported to the Potchefstroom Veterinary Laboratory for further identification to ensure uniformity in identification methods.

3.6 Bacterial identification

Once at the laboratory, settle plates were incubated at 37±2°C for a period of 48 hours at which point the number of colony forming units were manually counted. In cases of mixed growth, each individual colony was sub-cultured onto non selective media (blood agar), creating the pure cultures needed for further processing.

The primary identification tests which included checks for haemolysis, catalase, oxidase, and Gram staining, were performed on each isolate. As per the standard operating procedure document VDS-M-09/BA (Mhlongo, 2018b), Gram staining was performed by initially preparing a smear of the pure bacterial colonies on a microscope slide with sterile diluent (Onderstepoort Biological Products-OBP) after which the smears were air dried and heat fixed. Crystal violet was used as a primary stain, followed by lugol's iodine to promote dye retention. The application of acetone alcohol, which acts as a decolorizer removed the purple crystal violet-iodine complex from the thin walled Gram-negative organisms, whilst the thick peptidoglycan layer enabled the Gram-positive bacteria to retain the primary dye. Gram staining was completed by the application of a safranin counter stain.

Following characterisation of isolates by means of Gram staining reactions, the Sensititre ARIS 2X system was used for identification up to a species level. The Sensititre ARIS 2X (Trek Diagnostics) is an automated bacterial identification system with built-in incubation and reading module that has the ability to automatically identify both Gram-negative and Gram-positive bacteria (SOP: VDS-M-14/BA) (Mhlongo, 2018a). This identification system utilizes 96-well plates with three sections which are able to identify 3 isolates. Each section comprises of 32 dried biochemical tests that are pre-dosed onto the wells of Gram-positive (GPID) or Gram-negative (GNID) identification plates. A detailed procedure, outlined in Standard Operating Procedure document VDS-M-14/BA (Mhlongo, 2018a), entails preparation of a bacterial suspension using sterile demineralized water (Thermo Scientific Sensititre™, Remel Inc.) and then adjusting to a 0.5 McFarland Standard. After inoculating the wells with the

bacterial suspension, 3 drops of mineral oil were added to selected wells on both Gram-positive and Gram-negative plates. Once done, the wells were covered with an adhesive seal then incubated at 35ºC in ARIS 2X. After incubation, the fluorescent indicator (which had been mixed along with the media) allowed for automatic fluorometric reading and consequent conversion thereof into identification at a species level following computer analysis (Tang et al., 2013, O'Hara, 2005, Mahon et al., 2018). Bacterial identification was done for all collected samples.

Where organisms could not be identified more specifically than to a genus level, the identified genus was listed followed by the word 'species'. Furthermore because each genus is comprised of a large number of species, it could not be assumed that these isolates belonged to the same species as the other isolates within the same genus that could be identified more specifically. These organisms were thus counted as a separate species within each genus. For example, three of the streptococcal isolates could not be identified specifically and were thus simply listed as '*Streptococcus* species'. These 3 organisms were then considered to be the 8th species within the *Streptococcus* genus.

3.7 Antibiotic susceptibility testing

Isolates stored at -80ºC were sub-cultured on blood agar to obtain 24h pure cultures. Antibiograms were performed by preparing 0.85% saline-bacterial suspensions matching the 0.5% McFarland standard. The suspension was inoculated onto Mueller-Hinton agar or Mueller-Hinton with 5% sheep blood agar using a sterile swab, depending on the species of isolate. Appropriate antibiotic discs were selected based on the guidelines set out by the Clinical and Laboratory Standard Institute (CLSI). These guidelines were developed to aid with "selecting the correct and relevant antibiotic discs to use for various bacterial isolates causing different animal diseases" (VDS-M-06/UH)(Sizana, 2018).Various combinations of kanamycin (30µg), amoxicillin/clavulanic acid (30 µg), cephalothin (30 µg), enrofloxacin (5 µg), sulfisoxazole (300 µg), trimethoprim sulpha (25 µg), erythromycin (15 µg), tetracycline (30 µg), gentamicin (10 µg) and ampicillin (10 µg) were used in this study depending on what was appropriate for each species. The selected discs were then placed onto inoculated agar using a disc dispenser (Oxoid). The quality control of the test was assured using an appropriate ATCC strain following the same test procedure.

The plates were then incubated in an inverted position at 35°C±2°C for at least 18 hours. After incubation, zones of inhibition around the antibiotic discs were measured and results were interpreted as either 'sensitive', 'intermediate' or 'resistant' according to the breakpoints prescribed in the CLSI standard. Since it was not possible to perform antibiotic susceptibility testing on all isolates, as a way of minimizing unnecessary repetition, if more than one CFU of a species was isolated on a single plate, the isolates were assumed to belong to the same strain and thus only one was tested for its antibiotic susceptibility.

3.8 Data analysis

All results were analysed using simple descriptive statistics. The average deposition rate for each procedure/facility was calculated by dividing the total collection time by the total number of colony forming units to give the time per CFU.

For the expected contribution that each person made to the bioload per time period, the sum of the procedure duration multiplied by the occupants per procedure provided the total occupancy time for the facility. The total number of commensal organisms isolated at the facility was then divided by this number. This is summarized by the equation:

$$Bioload\ per\ person = \frac{Total\ number\ of\ commensal\ organisms\ isolated\ \ at\ facility}{\sum Procedure\ \ duration\ \mathrm{X}\ number\ of\ occupants\ per\ procedure}$$

4. RESULTS

4.1 Settle plate results

4.1.1 Species distribution

A total of 45 settle plates were placed in the above-mentioned veterinary establishments. The total collection time of 843 minutes yielded 487 bacterial isolates (53 species) resulting in an average deposition rate of 1 colony forming unit every 1 minute 44 seconds. The vast majority of cultured species, were Gram-positive in nature (81.3%), with organisms from the genera *Micrococcus* (35.3%), *Staphylococcus* (20.7%), *Corynebacterium* (14.4%), *Bacillus* (10.6%) and *Streptococcus* (8.6%) being most commonly represented. Gram-negative isolates comprised mainly of the *Moraxella* (25.3%), *Chryseobacterium* (19.8%), *Acinetobacter* (13.2%), *Yersinia* (9.9%) and *Sphingomonas* (6.6%) genera along with 11 others in less significant proportions. A detailed breakdown is available in Figures 4-1 and 4-2.

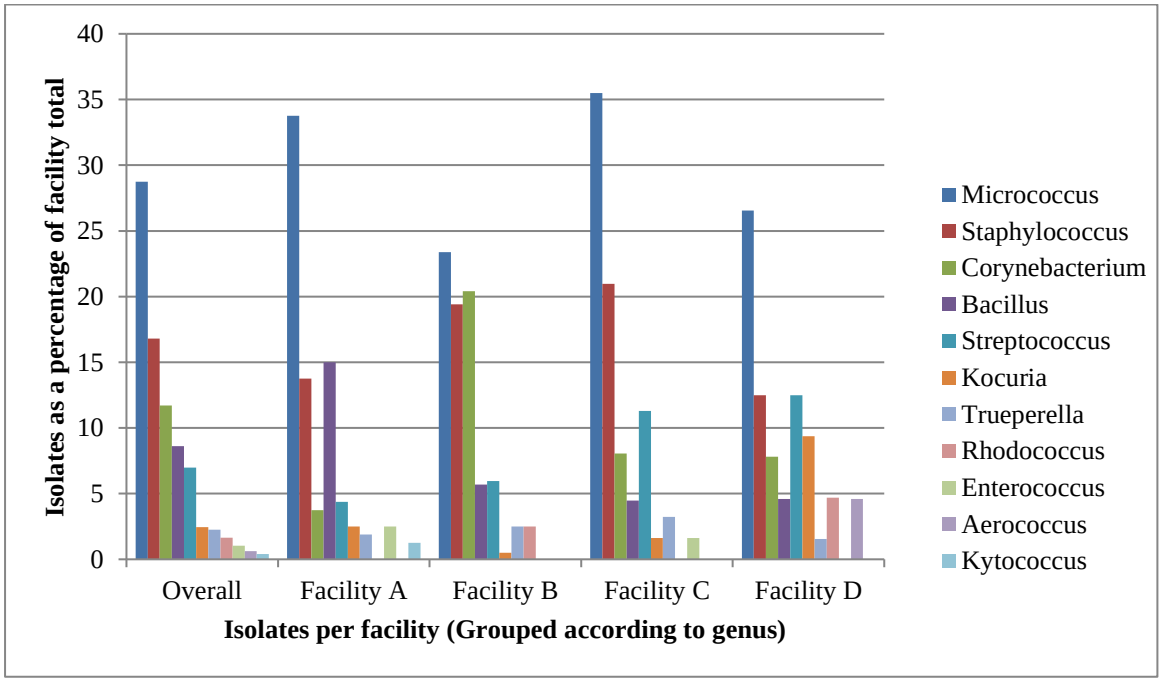

Figure 4-1: Gram-positive isolates collected at each facility
Grouped according to genus with isolates depicted as a percentage of facility total

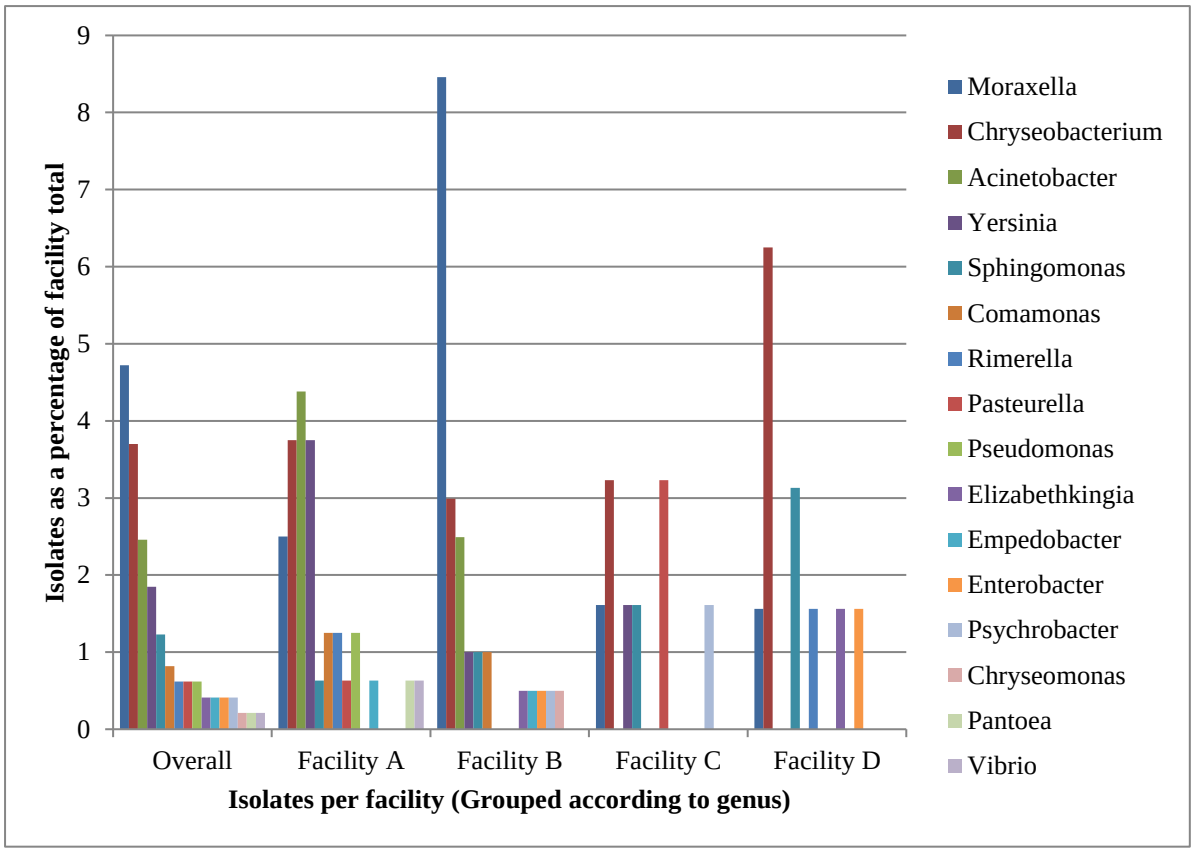

Figure 4-2 Gram-negative isolates collected at each facility

Grouped according to genus with isolates depicted as a percentage of facility total

4.1.1.1 Facility A

A total of 12 settle plates were collected over a span of 3 surgical days at Facility A. Procedures, which included both canine and feline patients, had an average duration of 17 minutes. A total of 160 colony forming units (CFU), comprising of 33 species, were isolated. Similar to overall data, at 77.5%, Gram-positive organisms were predominant, with the same genera accounting for the largest proportion of the isolates, though be it in slightly different proportions. The *Acinetobacter*, followed closely by the *Yersinia* and *Chryseobacterium* genera accounted for over 55% of the Gram-negative isolates, with the remainder being formed by the 9 other genera of bacteria. When looking at the total data collected from this facility, an average deposition rate of one CFU every 1 minute 18 seconds can be calculated. When looking at individual procedure data however, deposition rates varied from 1CFU per 30 seconds to 1 CFU every 2 minutes 20 seconds, despite the fairly consistent observable surgical conditions. Great variation was furthermore noted in the species which were isolated on the individual settle plates.

Micrococcus luteus was the only species to appear on all 12 plates, this was followed by the *Bacillus* species which was noted on 7 plates. No other species was isolated from more than 4 settle plates, despite the sampling of consecutive procedures.

4.1.1.2 Facility B

At Facility B, settle plates were placed during twelve canine sterilization procedures. A total operative time of 196 minutes were sampled, yielding 201 colony forming units, representing 36 species. As at Facility A, at 80.6%, Gram-positive organisms represented the vast majority of isolates, with *Micrococcus*, *Staphylococcus* and *Corynebacterium* forming just shy of 80% of these CFU's. The *Moraxella* genus represented 43.6% of Gram-negative isolates with 10 additional genera accounting for the remainder. Despite significant differences in deposition rates between individual plates (ranging from 1 colony every 36 seconds to one every 3 minutes with an average of 1CFU every 59 seconds), great overlap was seen on samples collected on the same day.

4.1.1.3 Facility C

Settle plates were placed for 13 procedures at Facility C, resulting in a total collection time of 278 minutes. A total of 62 colony forming units, comprising of 17 species were isolated. Once again, isolated bacteria were mainly Gram-positive in nature (87.1%), with the trend in genera reflecting collected data as a whole. Of the Gram-negative isolates that were cultured, no one species was predominant. Deposition rates were significantly slower at this practice when compared to other locations sampled. Time per colony forming unit ranged from 2 minutes 12 seconds to 22 minutes, averaging out at 4 minutes 36 seconds.

4.1.1.4 Facility D

At Facility D, the theatres belonging to D1 (i.e. the HEPA equipped theatres) yielded between 4 and 10 colony forming units per plate whilst the theatres forming part of D2 (i.e. non HEPA equipped theatres) yielded between 8 and 11 CFU per pate. The average deposition rate for the facility as a whole was 1CFU every 2min 30sec. In each case the settle plate placed directly next to the patient yielded a higher plate count than the corresponding plate placed on the anaesthetic machine. Despite settle plates being placed approximately 1-2m apart, a large variation in species between corresponding plates was evident. It was furthermore noted that at 18 species, the non HEPA equipped operating suites (D2) had a much larger species distribution than the 10 encountered in the HEPA equipped theatres (D1). The ratio of Gram-

positive to Gram-negative organisms, in both the HEPA and non HEPA filtered operating suites were consistent with data collected from other veterinary practices.

4.1.2. Expected origin of organisms

In order to evaluate how the above mentioned surgical practices at each facility could be influencing the sampled bacterial population, the natural habitat of each of the sampled species was researched, allowing isolates to be further subcategorized into commensals (i.e. those that form part of the normal microflora of humans/ small animals) and non-commensals. Organisms which are occasionally isolated as commensals, but commonly found in the environment, were categorized as non-commensals. Based on this categorization the results were as follows: at 53.1%, Facility D had the lowest proportion of commensals (50.0% at D1 and 55.6% at D2), this was followed by Facility B at 59.7%, Facility A at 63.1% and lastly with the highest percentage of commensals would be Facility C at 74.2%. Because of the vastly different protocols between facilities, these figures cannot however be directly compared.

As discussed earlier, the number of personnel, the time they spent in the operating room, as well as the surgical attire that they wear has a direct impact on the bioaerosol load. Thus, in order to compare the effects of the various combinations of surgical attire between facilities, the commensal bioload per person per minute was calculated. Following these calculations it was determined that Facility D had the lowest commensal bioload per person per time period. Practice C at 1.2x the number of commensals per occupancy time than practice D, was the next lowest. This was followed by practice B at 2.6x and finally practice A at 5.0x that of practice D. These results are summarized in Table 4-1 below.

Table 4-1: Summary of organisms isolated per facility

		Facility						
		Overall	**A**	**B**	**C**	**D**	**D1**	**D2**
Plates		45	12	12	13	8	4	4
Total Collection time (min)		843	209	196	278	160	80	80
Deposition Rate (Time/ CFU)		1min 44sec	1min 18sec	0min 59sec	4min 36sec	2min 30sec	2min 51sec	2min 13sec
Species	**Total**	53	33	36	17	22	10	18
	Commensal (%)	45.3	48.5	44.4	52.9	36.4	40.00	38.9
Isolates	**Total**	487	160	201	62	64	28	36
	Commensal (%)	61.8	63.1	59.7	74.2	53.1	50.0	55.6
Total occupancy time (min)		1986	408	929	766	720	320	400
Bioload per person		0.15	0.25	0,13	0.06	0.05	0.04	0.05
Key: CFU – Colony forming unit								

4.1.3 Pathogenicity of isolated organisms

When the isolates were evaluated in terms of their pathogenicity it was found that 37.2% belonged to species that have been previously implicated in small animal surgical site infections.

These organisms included: *Micrococcus luteus*, *Micrococcus* species, *Pseudomonas* species, *Streptococcus* species, *Enterococcus faecalis*, *Enterobacter cloacae*, *Staphylococcus pseudintermedius*, *S. aure*us, coagulase-positive staphylococci and coagulase-negative staphylococci.

4.2 Antimicrobial susceptibility testing results

Following completion of sample collection, antibiograms were performed on remaining viable organisms. As a cost saving measure and to avoid unnecessary repetition, in cases where more than one CFU of the same bacterial species was isolated on one plate, all isolates were treated as belonging to a single strain and thus only a single CFU was selected for further antimicrobial susceptibility testing.

Erroneously the isolates collected at Facility D were discarded prior to antimicrobial susceptibility testing by the laboratory and thus all data collected at Facility D will be excluded for this portion of the data analysis/ discussion.

4.2.1 Overall

During the course of this study 423 isolates representing 50 bacterial species were isolated at Facilities A, B and C. Antimicrobial susceptibility testing was performed on 102 of these colonies (24.1%), covering 41 species (82.0%) (Table 4-2). If looked at in terms of total sample population (including facility D) it would only represent 20.9% of isolates (77.4% of species). Antimicrobial resistance was detected in 58.8% of the tested isolates and 80.5% of the tested species.

Table 4-2 Summary of Antimicrobial Susceptibility Results

Genus	Tested	Susceptible		Resistant	
		no.	%	no.	%
Acinetobacter	3	2	66.7	1	33.3
Bacillus	13	7	53.9	6	46.2
Chryseobacterium	4	3	75.0	1	25.0
Comamonas	2	0	0.0	2	100.0
Corynebacterium	8	7	87.5	1	12.5
Enterobacter	1	0	0.0	1	100.0
Enterococcus	2	0	0.0	2	100.0
Kocuria	3	2	66.7	1	33.3
Kytococcus	1	0	0.0	1	100.0
Micrococcus	14	8	57.1	6	42.9
Moraxella	6	2	33.3	4	66.7
Pantoea	1	0	0.0	1	100.0
Pseudomonas	1	0	0.0	1	100.0
Psychrobacter	1	1	100.0	0	0.0
Rhodococcus	1	0	0.0	1	100.0
Sphingomonas	1	0	0.0	1	100.0
Staphylococcus	25	7	28.0	18	72.0
Streptococcus	8	1	12.5	7	87.5
Trueperella	4	0	0.0	4	100.0
Vibrio	1	1	100.0	0	0.0
Yersinia	2	2	100.0	0	0.0

When evaluating antimicrobial resistance at a species and genus level, clusters of resistance or lack thereof to specific antimicrobials were evident among tested organisms.

4.2.2 Resistance trends grouped according to genus

The antimicrobial resistance trends per species are presented in Table 4-3, with a more detailed description of each species being discussed below.

4.2.2.2 Staphylococcus

A total of 25 organisms forming part of the *Staphylococcus* genus were tested, the largest number in any single genus. Of these organisms, 72.0 % were resistant to one or more of the 7 antibiotics against which they were tested. Of the organisms that were resistant, erythromycin resistance, at 61.1%, was most commonly encountered in this group; followed by tetracycline at 44.4%, and trimethoprim/sulpha at 27.8%. Resistance to kanamycin and enrofloxacin was infrequent, with only one organism displaying resistance to each. All organisms were susceptible to amoxicillin/clavulanic acid and cephalothin. When the criteria of a multidrug resistant organism, namely organisms which are "resistant to one or more classes of antimicrobial agents" as defined by the CDC (Siegel et al., 2017) is applied to the obtained results, it was found that multidrug resistance was relatively scarce among these organisms. Only 27.8% of tested staphylococcal organisms were resistant to two antimicrobials, whilst a single isolate, namely *S. saprophyticus,* was resistant to four. The remainder and thus majority of the isolates were resistant to a single drug only.

4.2.2.3 Streptococcus

Among the *Streptococcus* genus, only 12.5% of the organisms were completely susceptible to all 7 of the antimicrobials tested. Resistance to kanamycin was overwhelmingly prevalent with all tested organisms, except one, being resistant to this active ingredient. This was true for all streptococcal isolates despite consisting of 5 species, collected over 6 sample collection days which were spread out over 2 months, in 3 practices. Tetracycline resistance, being present in 3 of the 8 isolates, was also fairly common. Resistance to enrofloxacin was found in the same number of isolates however to a lesser degree, with organisms only displaying an intermediate degree of resistance. Trimethoprim/sulpha and erythromycin resistance was additionally encountered but in a fewer number of isolates. When compared to the staphylococcal isolates, multidrug resistance was present in a larger number of isolates, i.e. 71.4%, with no single organism being resistant to more than 4 antimicrobials.

4.2.2.4 Micrococcus

A total of 14 *Micrococcus luteus* isolates, the most widely encountered organism, were tested for their antimicrobial susceptibility. Of these isolates, more than half (ie 57.1%) showed no antimicrobial resistance. Unlike the *Staphylococcus* and *Streptococcus* genera however, multidrug resistance was very rare in this group of organisms, with only a single isolate being

resistant to both cephalothin and sulfisoxazole. The remaining organisms were resistant to only one or the other, with sulfisoxazole resistance being the most frequently encountered.

4.2.2.5 Bacillus

As with the *Staphylococcus, Streptococcus* and *Micrococcus* genera where resistance to a particular type of antibiotic was prevalent, the same holds true for the *Bacillus* genus, except in this case, a multidrug resistance pattern was evident. Of the 13 isolates tested, only 7 were completely susceptible to all tested antimicrobials. Of the isolates that were resistant, 85.7% were resistant to both amoxicillin/clavulanic acid and cephalothin, despite some samples being collected over a month apart and in more than one facility. A further 60% of these organisms were additionally resistant to tetracycline. Kanamycin and enrofloxacin resistance was additionally detected, though infrequently. A single isolate was resistant to all 5 of these aforementioned active ingredients.

4.2.2.6 Moraxella

Though the *Moraxella* group of organisms displayed a less distinguishable pattern of antimicrobial resistance, possible site specific resistance trends may have emerged if more isolates were tested. The single isolate from Facility C was completely susceptible, as was one isolate from Facility B. The remaining 2 isolates from Facility B both displayed an intermediate level of resistance to enrofloxacin, with no multidrug resistance being detected. Both tested isolates from Facility A on the other hand were multidrug resistant. One isolate was resistant to tetracycline and sulfisoxazole, whilst the other displayed some degree of resistance to all 6 active ingredients tested.

4.2.2.7 Enterococcus

Unlike *Moraxella* where multidrug resistance was encountered in the minority of isolates, the same did not hold true for the *Enterococcus* genus. Though only 2 isolates consisting of 2 species namely *E. durans* and *E. faecalis* were tested, both showed a significant degree of resistance. *E. faecalis* displayed an intermediate degree of resistance to tetracycline and was completely resistant to kanamycin, enrofloxacin and amoxicillin clavulanic acid. *E. durans*, in addition to the resistance profile seen in *E. faecalis*, was also resistant to ampicillin and erythromycin, thus being resistant to 7 of the 8 tested antimicrobials.

4.2.2.8 Enterobacter

Though the level of antimicrobial resistance present in the *Enterococcus* genus was concerning, our tests revealed that the isolates were still susceptible to at least one antimicrobial. This was not the case in the *Enterobacter cloacae* isolate that was tested. This organism was completely resistant to all 6 antimicrobials (amoxicillin/clavulanic acid, cephalothin, enrofloxacin, sulfisoxazole, tetracycline and gentamicin). Unfortunately only one isolate was collected during the course of the study and thus no comparison exists to determine the frequency of this finding in this species.

4.2.2.9 Yersinia

Related at a 'familial level' the results of the Yersinia genus are a stark contrast to those of the *Enterobacter* genus - neither of the 2 isolates displayed antimicrobial resistance to any degree.

4.2.2.10 Corynebacterium

Of the genera where multiple (more than 2) isolates were tested, the *Corynebacterium* genus displayed the lowest proportion of resistant organisms. Of the 8 isolates tested, 87.5% were completely susceptible to all antimicrobials. Only one isolate, namely a *Corynebacterium pseudotuberculosis* isolate at Facility A, showed any degree of resistance – in this case intermediate resistance to enrofloxacin.

4.2.2.11 Trueperella

At the opposite end of the spectrum would be *Trueperella pyogenes*. Of all of the species in which four or more isolates were tested, *T. pyogenes* was the only species in which 100% of organisms displayed resistance.

4.2.2.12 Overall resistance trends

Table 4-3 Antimicrobial resistance trends grouped according to genus

Genus	No. of Isolates tested	Percentage of tested isolates that were resistant to each antimicrobial										Resistant Isolates[a]	Multidrug resistance[b]
		Kanamycin	Amoxicillin/ clavulanic acid	Cephalothin	Enrofloxacin	Sulfisoxazole	Trimethoprim/ sulpha	Erythromycin	Tetracycline	Gentamycin	Ampicillin		
		n %	n %	n %	n %	n %	n %	n %	n %	n %	n %	n	n
Acinetobacter	3	- NT	0 0,0	1 33,3	0 0,0	0 0,0	- NT	- NT	0 0,0	0 0,0	- NT	1	0
Bacillus	13	2 15,4	5 38,5	5 38,5	1 7,7	0 0,0	- NT	- NT	3 23,1	- NT	- NT	6	5
Chryseobacterium	4	- NT	0 0,0	0 0,0	0 0,0	1 25,0	- NT	- NT	0 0,0	- 0,0	- NT	1	0
Comamonas	2	- NT	1 50,0	1 50,0	0 0,0	0 0,0	- NT	- NT	0 0,0	1 50,0	NT	2	1
Corynebacterium	8	0 0,0	0 0,0	0 0,0	1 12,5	0 0,0	- NT	- NT	0 0,0	- NT	- NT	1	0
Enterobacter	1	- NT	1 100,0	1 100,0	1 100,0	1 100,0	- NT	- NT	1 100,0	1 100,0	- NT	1	1
Enterococcus	2	2 100,0	0 0,0	1 50,0	2 100,0	2 100,0	- NT	1 50,0	2 100,0	- NT	1 50,0	2	2
Kocuria	3	1 33,3	0 0,0	1 33,3	1 33,3	0 0,0	1 33,3	1 33,3	0 0,0	- NT	- NT	1	1
Kytococcus	1	0 0,0	0 0,0	0 0,0	0 0,0	1 100,0	- NT	- NT	0 0,0	- NT	- NT	1	0
Micrococcus	14	1 7,1	0 0,0	2 14,3	0 0,0	4 28,6	- NT	- NT	0 0,0	- NT	- NT	6	1
Moraxella	6	1 16,7	1 16,7	1 16,7	3 50,0	2 33,3	- NT	- NT	2 33,3	0 0,0	- NT	4	2
Pantoea	1	- NT	0 0,0	0 0,0	0 0,0	1 100,0	- NT	- NT	0 0,0	1 100,0	- NT	1	1
Pseudomonas	1	0 0,0	0 0,0	1 100,0	0 0,0	0 0,0	- NT	- NT	0 0,0	- NT	- NT	1	0

Genus	No. of Isolates tested	Percentage of tested isolates that were resistant to each antimicrobial										Resistant Isolates[a]	Multidrug resistance[b]
		Kanamycin	Amoxicillin/ clavulanic acid	Cephalothin	Enrofloxacin	Sulfisoxazole	Trimethoprim/ sulpha	Erythromycin	Tetracycline	Gentamycin	Ampicillin		
		n %	n %	n %	n %	n %	n %	n %	n %	n %	n %	n	n
Psychrobacter	1	- NT	0 0,0	0 0,0	0 0,0	0 0,0	- NT	- NT	0 0,0	0 0,0	- NT	0	0
Rhodococcus	1	1 100,0	0 0,0	1 100,0	1 100,0	0 0,0	- NT	- NT	0 0,0	- NT	1 NT	1	1
Sphingomonas	1	- NT	0 0,0	0 0,0	0 0,0	0 0,0	- NT	- NT	0 0,0	1 100,0	- NT	1	0
Staphylococcus	25	1 4,0	0 0,0	0 0,0	1 4,0	- NT	5 20,0	11 44,0	8 32,0	- NT	- NT	18	6
Streptococcus	8	7 87,5	0 0,0	0 0,0	3 37,5	- NT	2 25,0	1 12,5	3 37,5	- NT	- NT	7	4
Trueperella	4	1 25,0	0 0,0	0 0,0	2 50,0	0 0,0	- NT	- NT	1 25,0	- NT	- NT	4	0
Vibrio	1	- NT	0 0,0	0 0,0	0 0,0	0 0,0	- NT	- NT	0 0,0	0 0,0	- NT	0	0
Yersinia	2	- NT	0 0,0	0 0,0	0 0,0	0 0,0	- NT	- NT	0 0,0	0 0,0	- NT	0	0

[a] Number of tested isolates that are resistant to at least one antimicrobial

[b] Number of isolates that are resistant to two or more antimicrobial classes

NT indicates not tested for that specific antimicrobial

4.2.3 Resistance in organisms previously implicated in SSI's

Of the 41 species for which antimicrobial resistance testing was done, 7 have been previously implicated in veterinary surgical site infections. The results for these isolates are available in table 4-4 below.

Table 4-4 Antimicrobial resistance detected in species that have previously been implicated in veterinary surgical site infections

Genus	No. of Isolates tested	Percentage of tested isolates that were resistant to each antibiotic										Resistant Isolates[a]	Multidrug resistance[b]
		Kanamycin	Amoxicillin/ clavulanic acid	Cephalothin	Enrofloxacin	Sulfisoxazole	Trimethoprim/ sulpha	Erythromycin	Tetracycline	Gentamycin	Ampicillin		
		n %	n %	n %	n %	n %	n %	n %	n %	n %	n %	n	n
Enterobacter cloacae	1	- NT	1 100.0	1 100.0	1 100.0	1 100.0	- NT	- NT	1 100.0	1 100.0	- NT	1	1
Enterococcus faecalis	1	1 100.0	0 0.0	0 0.0	1 100.0	1 100.0	- NT	0 0	1 100.0	- NT	0 0	1	1
Micrococcus luteus	14	1 7.1	0 0.0	2 14.3	0 0.0	4 28.6	- NT	- NT	0 0.0	- NT	- NT	6	1
Pseudomonas species[c]	1	0 0.0	0 0.0	1 100.0	0 0.0	0 0.0	- NT	- NT	0 0.0	- NT	- NT	1	0
Staphylococcus aureus	2	0 0.0	0 0.0	0 0.0	0 0.0	- NT	0 0.0	0 0.0	0 0.0	- NT	- NT	0	0
Staphylococcus pseudintermedius	5	0 0.0	0 0.0	0 0.0	0 0.0	- NT	1 20.0	1 20.0	0 0.0	- NT	- NT	2	0
CONS[c]	1	0 0.0	0 0.0	0 0.0	1 100.0	- NT	0 0.0	0 0.0	0 0.0	- NT	- NT	1	0

[a] Percentage of tested isolates that are resistant to at least one antibiotic

[b] Number of isolates that are resistant to two or more antibiotic classes

[c] Could not be identified more specifically

NT indicates not tested for that specific antibiotic

CONS – Coagulase-negative *Staphylococcus*

4.2.4 Results grouped according to facility

When looking at each practice individually, the results were as follows.

Table 4-5 Antimicrobial susceptibility results, grouped according to facility

Genus	Overall			Facility A			Facility B			Facility C		
	Tested	Resistant		Tested	Resistant		Tested	Resistant		Tested	Resistant	
	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%
Acinetobacter	3	2	66.7	0	-	-	3	1	33.3	0	-	-
Bacillus	13	8	61.5	8	5	62.5	5	1	20.0	0	-	-
Chryseobacterium	4	3	75.0	2	1	50.0	1	0	0.0	1	0	0.0
Comamonas	2	0	0.0	1	1	100.0	1	1	100.0	0	-	-
Corynebacterium	8	7	87.5	1	1	100.0	6	0	0.0	1	0	0.0
Enterobacter	1	0	0.0	0	0	-	1	1	100.0	0	-	-
Enterococcus	2	0	0.0	2	2	100.0	0	-	-	0	-	-
Kocuria	3	2	66.7	1	0	0.0	1	1	100.0	1	0	0.0
Kytococcus	1	0	0.0	1	1	100.0	0	-	-	0	-	
Micrococcus	14	8	57.1	5	2	40.0	5	3	60.0	4	1	25.0
Moraxella	6	2	33.3	2	2	100.0	3	2	66.7	1	0	0.0
Pantoea	1	0	0.0	1	1	100.0	0	-	-	0	-	-
Pseudomonas	1	0	0.0	1	1	100.0	0	-	-	0	-	-
Psychrobacter	1	1	100.0	0	-	-	1	0	0.0	0	-	-
Rhodococcus	1	0	0.0	0	-	-	1	1	100.0	0	-	-
Sphingomonas	1	0	0.0	1	1	100.0	0	-	-	0	-	-
Staphylococcus	25	7	28.0	9	7	77.8	14	10	71.4	2	1	50.0
Streptococcus	8	1	12.5	3	3	100.0	4	3	75.0	1	1	100.0
Trueperella	4	0	0.0	1	1	100.0	2	2	100.0	1	1	100.0
Vibrio	1	1	100.0	1	0	0.0	0	-	-	0	-	-
Yersinia	2	2	100.0	1	0	0.0	1	0	0.0	0	-	-
Overall (isolates)	102	59	57.8	41	29	70.7	49	26	53.1	12	4	33.3
Overall (species)	41	33	80.5	27	24	88.9	27	19	63.0	9	4	44.4

4.2.4.1 Facility A

At 25.6% of the samples and 81.8% of the isolated species, the antimicrobial testing done on isolates collected at this practice were the most extensive and representative. Testing a large number of species came at the expense of testing multiple isolates of the same species, thus of the 27 species tested only 7 species were tested more than once.

At 70.7% of the tested samples, representing 88.9% of the tested species, the antimicrobial resistance encountered at this practice was high in comparison to the other facilities. What is more concerning however, is the degree of multidrug resistance encountered. Of the isolates tested, 34.1% were resistant to at least two antibiotics, with some organisms displaying resistance to in excess of 4, and in some cases up to 7, antimicrobials.

An isolate of *Moraxella catarrhalis* for example was not sensitive to any of the 6 antimicrobials against which it was tested – being only intermediately susceptible to amoxicillin/ clavulanic acid and enrofloxacin and completely resistant to kanamycin, cephalothin, sulfisoxizole and tetracycline. Despite being sensitive to amoxicillin/clavulanic acid, an *Enterococcus durans* isolate had an intermediate level of resistance to one antimicrobial (enrofloxacin) and complete resistance to the remaining 6 (kanamycin, cephalothin, sulfisoxazole, erythromycin, tetracycline and ampicillin) against which it was tested.

4.2.4.2 Practice B

Just shy of a quarter (24.4%) of the isolates collected at Facility B, representing 75% of the species, were tested for their antimicrobial susceptibility. Unlike the samples tested at Facility A, a larger proportion of species were tested more than once, thus despite testing a larger number of isolates, the same number of species (i.e. 27) were represented. This allowed for the slightly better evaluation of the overlap in susceptibility patterns within a species collected at the same practice but at different times. A spectrum of results was seen.

Resistance was not encountered in any of the tested organisms belonging to the *Corynebacterium* genus (6 isolates). The results were not as consistent within the *Staphylococcus* genus however, as only 2 isolates of each species were tested. Like the *Corynebacterium* isolates, both *Staphylococcus aureus* isolates were completely susceptible to all antimicrobials. *Staphylococcus* group G isolates on the other hand were resistant to a single antimicrobial (namely kanamycin). Having been collected on the same day these isolates could potentially belong to the same strain. Despite being collected on the same day, the *S. saprophyticus* isolates did not display identical resistance patterns, but overlap was noted: one isolate was resistant to erythromycin and tetracycline, whilst the other was additionally resistant to kanamycin and trimethoprim/sulpha. No overlap was seen within the *S. pseudintermedius* isolates despite being collected on the same day. A further two isolates could not be identified more specifically than belonging to the *Staphylococcus* coagulase-negative

group of organisms; considering that no overlap in resistance patterns were seen the possibility exists that these two isolates belonged to different species or different strains within the same species. Two different strains of *Trueperella pyogenes* were isolated on the same day, with each being resistant to a single, but different antimicrobial. A significant difference was noted between the two *Streptococcus pneumoniae* isolates: one was completely susceptible to all antimicrobials whilst the other was intermediately resistant to kanamycin and enrofloxacin and completely resistant to erythromycin. Of the remaining organisms where multiple isolates were tested – namely *Acinetobacter lwoffii*, *Bacillus cereus, Micrococcus luteus, Moraxella osloensis* and *Staphylococcus warneri*, haphazard antimicrobial susceptibility/ resistance patterns were noted indicating the presence of multiple strains within each species.

Despite having a lower proportion of organisms that displayed multi-drug resistance (20.4% vs. 34.1%), the number of isolates that were resistant to more than 4 antimicrobials was slightly higher in Facility B than in Facility A. (6.1% vs. 4.9%). At this practice a *Staphylococcus saprophyticus* isolate was resistant to kanamycin, trimethoprim/sulpha, erythromycin and tetracycline. Apart from tetracycline, *Kocuria rosea* was additionally resistant to cephalothin and enrofloxacin. An *Enterobacter cloacae* isolate however was the organism that displayed the largest degree of resistance - being resistant to all 6 antimicrobials tested. What is important to note is that unlike at Facility A where the all of the organisms that were resistant to four or more antimicrobials displayed intermediate susceptibility to some of the antibiotics, only complete resistance was present at Facility B.

4.2.4.3 Facility C

Only 12 isolates originating from this practice were tested for their antimicrobial susceptibility. Of these organisms, only 4 could be characterized as antimicrobial resistant: *Trueperella pyogenes* and *Micrococcus luteus* were resistant to cephalothin and kanamycin respectively, whilst *Staphylococcus epidermidis* and *Streptococcus dysgalactiae* were multidrug resistant (erythromycin and tetracycline, and trimethoprim/sulpha, tetracycline and kanamycin respectively). The remaining 8 organisms, including the three other isolates of *Micrococcus luteus,* were susceptible to all antimicrobials tested. When compared to the other two establishments, Facility C had the lowest degree of resistance, both in terms of the proportion of isolates and proportion of species.

5. DISCUSSION

5.1 Overview

As can be expected, a surgical site infection can have devastating effects on the patient, not only leading to elevated pain levels and delayed wound healing, but it can furthermore lead to increased morbidity, loss of function and in some cases even result in the death of the patient (Darouiche, 2016, Badia et al., 2017). What is often overlooked, however, is that the impact of surgical site infections extends beyond the patient itself. Apart from the financial and emotional burden placed on the owners and veterinary staff, the treatment (or apparent prevention) of SSI's can result in the misuse of antimicrobial drugs.

Considering the frequency with which canine and feline procedures are performed in the average veterinary practice and that surgical site infections are reported in 2-6% of clean procedures (Eugster et al., 2004, Spohrc et al., 2012, Turk et al., 2015, Vasseur et al., 1988, Shales, 2012), the large scale use of antimicrobial agents for a condition which may otherwise be preventable, may serve to further propagate antimicrobial resistance. With antimicrobial resistance being classified among the most important global health threats (WHO, 2019b), a wide scale multidisciplinary approach which aims to safe guard these vital drugs should be implemented. This includes evaluating conditions in individual veterinary practices which might be contributing to surgical site infection rates and consequently leading to the unnecessary use of antimicrobials. By placing settle plates in 4 veterinary facilities during routine canine and feline sterilization procedures, this study aimed to document the bacterial bioaerosol load present in veterinary theatres during routine clean procedures and evaluate the potential factors which might be contributing to both the sampled composition and load. The isolates were then further assessed for their antimicrobial susceptibility patterns to gain a better understanding of resistance patterns present in the average practice.

5.2 Method selection

Both active and passive sampling methods can be used to determine the bioaerosol load (Kasdekar et al., 2016). Whilst active sampling measures the number of organisms per volume of air sampled, passive sampling on the other hand gives an indication of the number of organisms that will deposit onto a surface (Tršan et al., 2019). Because this study focused on

airborne bacteria that could settle into the incision site, passive sampling by means of settle plates was deemed to be the more appropriate method. As with other studies which evaluated airborne contamination in theatres or clean rooms (Tršan et al., 2019, Tshokey et al., Naik et al., 2018), blood agar, a non-selective medium which allows for the isolation and further culture of numerous organisms (Merck, N.D.) was used for this study.

5.3 Isolated organisms

5.3.1. Classification of the Organisms predominance

In this study, the most evident trend amongst all veterinary theatres sampled would be the predominance of Gram-positive organisms. The 81.3% Gram-positive result closely reflects the 78% obtained by Tršan et al. (2019). In their study, a total of 9 519 samples (of which 7 257 used the blood agar settle plate method) were collected over a period of 5 years for the purpose of monitoring the environment within a hospital pharmacy cleanroom (i.e. where provisions are made to reduce particulate matter). Sudharsanam et al. (2008) similarly found the total Gram-positive counts to be higher in their study which evaluated the indoor air quality in hospitals in India. Additionally, in an article evaluating the effect of environmental parameters on the survival of airborne infectious agents, Tang (2009) states that both European and American based studies found Gram-positive organisms to be the predominant bacteria in an indoor environment.

Gram-positive organisms form part of the natural microbiota of skin and mucous membranes of humans and animals (Tolabi et al., 2019). It has been estimated that approximately 10% of the 30 000 – 40 000 skin cells that humans shed per minute (Sandle, 2014), carry microorganisms. This high rate of shedding serves as a source of environmental contamination, with the shed skin cells further serving as a nutrient substrate and source of moisture for these bacteria, thus allowing them to further replicate (Tršan et al., 2019).

Though data on the rate of shedding in companion animals is not as readily available, the presence of household pets have been shown to leave a distinct bioaerosol footprint (Barberán et al., 2015, Prussin and Marr, 2015). In a study evaluating the ecology of microscopic life in household dust, a total of 1142 indoor samples revealed that homes with dogs and cats had a significantly increased abundance of 56 and 24 bacterial genera respectively when compared

to homes without these pets (Barberán et al., 2015). The genera isolated include those that are commonly found in the mouths and faeces of pets (Barberán et al., 2015). The information was so predictive in fact, that when presented with the bacterial phylotypes alone, it was possible to predict the presence of a dog or cat occupant with 92% and 83% accuracy respectively (Barberán et al., 2015).

When the natural environment of each of the 53 species isolated in this study was evaluated, 45.3% were categorized as human and/or companion animal commensals, with all but 4 of those being classified as Gram-positive. Of these organisms only a single species, representing 3 isolates could be categorized as a 'small animal exclusive' commensal, with the remainder being either human exclusive, or in the majority of cases, commensals of both. It was therefore not possible to further separate isolates into those of human and those of animal origin when the same species may have originated from either. Overall despite only accounting for less than half of the isolated species, commensal bacteria represented 61.8% (301 out of 487) of the total number of isolated organisms.

The high rate of shedding of commensal organisms from either a person or the animal would explain the overall high proportion of Gram-positive organisms in the collected samples. The predominance of Gram-positive organisms is then further exacerbated by their superior ability to survive adverse environmental conditions (Tolabi et al., 2019).

When looking at the likely reason for the predominance of Gram-positive organisms, one can look at peptidoglycan, a component that is nearly universal amongst bacteria (Yadav et al., 2018). This exoskeleton-like polymer provides mechanical protection by preventing osmotic induced cell lysis and helps to preserve cell shape (Yadav et al., 2018, Salton and Kwang-Shin, 1996). The cell wall of Gram-positive bacteria consists of a relatively thick peptidoglycan layer of highly cross-linked peptide chains (Salton and Kwang-Shin, 1996) in which other cell wall polymers are embedded (Salton and Kwang-Shin, 1996). In contrast, the peptide chains in Gram-negative bacteria are only partially cross-linked, with the total peptidoglycan being much thinner (Salton and Kwang-Shin, 1996). Gram-negative bacteria additionally possess an outer envelope consisting of lipopolysaccharides (Salton and Kwang-Shin, 1996). Ultimately these modifications in peptide and chemical structures play a determining role in the organism's ability to survive external environmental challenges (Yadav et al., 2018). Consequently, as a whole, Gram-positive organisms are better able to withstand desiccation and can thus survive

in larger temperature and relative humidity ranges (Janning and in't Veld, 1994, Lemmen et al., 2004, Pettit and Lowbury, 1968).

Adaptations within individual Gram-positive species can provide a further competitive advantage, prolonging their survival time away from the host. The *Staphylococcus* genus for example, which commonly forms part of commensal organisms and which was frequently isolated in this study (17.04%), has the ability to rapidly alter their physiology and cellular activities in response to growth limiting challenges (Onyango and Alreshidi, 2018). Alterations in cell wall thickness, changes in the ratio of saturated to unsaturated fatty acids in the cell membrane as a way of maintaining membrane fluidity and the regulation of ribosomal proteins, are among the temperature induced modulations (Onyango and Alreshidi, 2018). They additionally possess osmoprotective mechanisms, can compensate for nutritional deprivation and have the ability to alter between homo- and heterogeneous population states depending on terrestrial stressors (Onyango and Alreshidi, 2018). This metabolic versatility has allowed these organisms to not only survive, but also to thrive in what could otherwise be classified as adverse environmental conditions.

5.3.2 Contributors to bioload

With the manner in which this study was conducted it was not possible to identify whether humans or animals were the source of the commensals. We are however able to speculate based on the findings. From information available from the World Health Organization (WHO), the Association of Surgical Technologists (AST) and the American Society for Anaesthesiologists (ASA), we know that exposed skin plays a major role in room contamination (Gawande et al., 2009, ASA, 2019a, AST, 2008). Considering that all of the procedures monitored followed single patients into the room at a time, together with drapes being applied albeit slightly differently, one can expect the level of shedding from the animals between procedures and facilities to be similar. Thus even though the contribution of animal origin commensals may not be negligible, when comparing between facilities it would be the person(s) in theatre and their level of surgical attire, that would be considered the major contributors to the bioload.

As shown earlier in table 3-1, Facility A consistently utilized examination gloves, surgical masks, head coverings and partial sterile surgical drapes during their surgical procedures. Facility B made use of surgical gloves, partial sterile surgical drapes, inconsistently used masks and never utilized scrub caps. Examination gloves were the only form of surgical attire utilized

by staff at Facility C, with non-sterile partial drapes being used to cover their patients which had been surgically prepared in the theatre itself. None of these facilities made use of surgical scrubs or gowns. These practices are in stark contrast to those implemented at Facility D where full surgical attire, including scrub suits, gowns, masks, head gear and gloves were utilized, in combination with full sterile patient drapes.

When looking purely at these practices, it would be expected that Facility D would have the lowest commensal bacterial load, followed by Facility A and then B, with Facility C having the highest commensal bioload per person. However, because the average procedure length and number of occupants varied greatly between the four veterinary establishments, the total number of commensals per practice could not be used as a sole comparator figure. To correct for this, the number of commensals per total occupancy time (i.e. the bioload per person) was calculated. These calculations, which served as a way of comparing the effect that personnel had on the bacterial bioload between facilities, yielded the following results: Facility D, as expected, had the lowest commensal bioload per person per time period. The other facilities however did not follow the expected trend. Instead Facility C, at 1.2x the number of commensals per occupancy time when compared to Facility D, was the next lowest. This was followed by Facility B at 2.6x and finally Facility A at 5x that of Facility D.

A possible explanation for these findings, which were essentially the opposite to what was expected, would be the extended survival of organisms in the environment and the consequent cumulative effect that room occupancy and consecutive procedures has on bacterial counts.

As discussed earlier, once present in the environment, some organisms can survive for extended periods of time. *Staphylococcus epidermidis*, an organism commonly isolated in this study for example, has been shown to remain viable in the environment for 5 days (Thompson et al., 2011). Though passive sampling indicates the number of organisms that would settle onto a surface during the collection time, it would be incorrect to assume that it is only indicative of organisms introduced into the environment during that same period. Instead, organisms introduced during preceding procedures, in some cases even days previously, may still be viable and thus contribute to the collected sample.

When re-evaluating the commensal bioload per person calculated earlier with this knowledge in mind, the results start to make sense. With approximately 10 procedures being performed on

a daily basis, 5 days a week, the cumulative effect of the theatre occupancy at Facility A would be quite high. This could explain the high commensal bioload per person despite the utilization of relatively better level of surgical attire. Facility B, which had an intermediate throughput at 4-5 daily procedures, had the second highest commensal bioload per person, whilst Facility C, which only performs approximately 2 procedures per day, had a commensal bioload only slightly higher than that of Facility D despite utilizing very few forms of surgical attire. Facility D, which had the lowest commensal bioload per person per occupancy time, could at least, in part be attributed to the low usage of the facility (once a month). This lead us to ultimately conclude that the level of contamination in veterinary surgical theatres evaluated, is linked to the total amount of time persons spend in theatre i.e. the greater the number of procedures, the more time a person has to contaminate the environment and the greater the consequent accumulation.

5.3.3. Further contributors to the bioload

The significant contribution that people make to the bioload of veterinary surgical theatres may be mitigated by the use of proper surgical attire as mentioned above. However, as mentioned earlier, despite these measures, staff can still shed approximately 10 000 squamous epithelial cells per person per minute (Al-Waked, 2010). The latter thus needs to be removed from theatres as they can accumulate and be re-suspended as a person moves through the environment. As a result further mitigation needs to be applied.

5.3.3.1 Recommended air conditions

In order to ensure that the air entering the facility does not serve as an additional source of pathogenic organisms, various bodies of authority, including the CDC, recommend that all air entering a surgical theatre, whether recirculated or fresh, pass through filters with a minimum 90% efficacy (Sehulster and Chinn, 2003, Chinn and Sehulster, 2003), with high efficiency particulate air (HEPA) filters, capable of removing 99.97% of particles larger than 0.3µm, being utilized in high risk areas (Chinn and Sehulster, 2003).

In an ideal setting, each theatre would be equipped with a HEPA filtration unit to filter all air entering the room, a minimum of 15-25 air changes would take place each hour to offset the continuous shedding of organisms from the patient and surgical staff (Owens and Stoessel,

2008, Mangram et al., 1999, Maheshwari, 2012, Singhal, 2018), and the room would have a positive pressure in relation to surrounding rooms to prevent the influx of organisms from relatively less clean to clean environments (Owens and Stoessel, 2008, Singhal, 2018).

At Facility D, the specific theatres equipped with HEPA filtration units (namely D1) had a lower absolute number of isolates (28 in comparison to 36), comprising of fewer species (10 in relation to 18) than the neighbouring theatres (D2).

Since HEPA air systems were not available at the remaining veterinary surgical theatres, facilities were reliant on open air ventilation. In a study entitled 'Natural Ventilation for the Prevention of Airborne Contagion' (Escombe et al., 2007) the authors concluded that "opening windows and doors maximizes natural ventilation so that the risk of airborne contagion is much lower than with costly, maintenance-requiring mechanical ventilation systems. Old-fashioned clinical areas with high ceilings and large windows provide greatest protection" (Escombe et al., 2007). Though sounding promising with regards to pathogen dilution, this study specifically focused on preventing the spread of tuberculosis in resource-limited settings. Considering that the highest tuberculosis load would be within the hospital where ill patients congregate (Escombe et al., 2007), the introduction of a continuous large volume of non-filtered air from an outside source would serve to dilute this accumulated pathogen. This same principle can however not as easily be extrapolated to theatre cleanliness. Surgical site infections are complex in nature with various bacterial species being implicated in the pathogenesis thereof. Leaving windows and doors open may serve to dilute some of the commensal organisms that are present, but at the same time can introduce additional environmental organisms as "outdoor air is thought to be the most important source of indoor micro flora" (Lina et al., 2019).

When looking at the three practices, they all had a somewhat different approach to managing air flow. For Facility A, the theatre door and windows remained closed whether or not the room was in use; while Facility B, in general, had 1 door and at times 3 doors left open with large amounts of movement; and lastly at Facility C, the theatre doors remained closed in 77% of procedures, with at least one door remaining open for the remainder of the day on a regular basis. For the situation created in Facility A, one can get the build-up of significant bioloads if the physical environment is not properly cleaned to remove the organisms. A partial solution would be to allow some air to enter to dilute the concentration of potentially pathogenic

organisms (Melhado et al., 2006, Memarzadeh and Xu, 2012, Faulkner et al., 2015) when the theatre is not in use, as seen with Facility B and C. For Facility C, we believe this was still ineffective as 74.2% of the organisms isolated were still categorized as human/ small animal commensals, perhaps due to insufficient turbulence when the dilution was meant to take place (WHO, 2016)(further discussion below). In contrast this was achieved to a slightly better effect in Facility B, which at 59.7%, had the lowest proportion of commensals of these three facilities.

In addition to the quality of air entering the facility, the movement and degree of turbulence within the room additionally alters the distribution of organisms (WHO, 2016). Indoor air particles take part in a deposition-resuspension cycle, the rate of which is dependent on particle size, relative humidity, degree of adhesion between the particle and the substrate, as well as ambient air currents (velocity and turbulence)(Mukai et al., 2009). Of these factors, the degree of air movement is most easily influenced by human activity. The air disturbance created by opening doors, moving equipment or from the movement of people themselves (Gizaw et al., 2016, Mukai et al., 2009, Tellier et al., 2019) can cause previously settled particles to dislodge and join the air stream (Mukai et al., 2009). The turbulence created can additionally allow, particularly the larger particles which settle more quickly, to remain suspended for longer periods of time (Mukai et al., 2009, Tellier et al., 2019).

The effect of re-suspension can be seen at Facility D whereby the organisms that deposited onto the plates more likely originated from re-suspended particles as a result of persons moving in the immediate environment, than from particles that have been suspended for an extended period of time, as these would have been removed by the HEPA filtration units.

Considering that the data collected during this study is dependent on the deposition of particles onto settle plates, it is unlikely that the collected isolates are representative of the total bioaerosol load, i.e. true bioload levels may be higher. This is particularly important in establishments, such as Facility B, in which a high degree of movement (both in terms of human activity as well as from open doors) was noted. Despite the implications of air currents on particle deposition, the affect thereof on particles settling into the wound and those settling onto the settle plate can be assumed to be equal and thus the data collected in this study is still considered adequate for its purpose.

The above mentioned results furthermore indicate that open ventilation is unlikely to be very effective in veterinary theatres, thereby necessitating other control measures. To overcome this, veterinary theatres without adequate mechanical ventilation systems need to keep open ventilation to a minimum; allow for turbulence when open ventilation is allowed (e.g. following completion of procedures for the day); and most importantly need to ensure the wearing of proper attire and implementation of mechanical cleaning to reduce the accumulation of organisms in the surgical suite environment.

5.3.3.2. Theatre cleaning

A typical theatre day should start with the preliminary cleaning of the operating room whereby all horizontal surfaces are damp-dusted. Immediate cleaning of all spills and biological waste should take place intra-operatively. Between procedures, the entire OR, including but not limited to all equipment and surfaces in the immediate vicinity of the operative area, all surfaces and pieces of equipment that have been in contact (whether direct or indirect) with the patient or bodily fluids, all visibly soiled areas, the anaesthetic equipment and all area's that have been touched by any staff members, including equipment and light switches should be cleaned. At the end of the day, or at least once every 24 hours terminal cleaning, in which all exposed surfaces including but not limited to lights, sinks, bins, and equipment wheels are disinfected, should take place (Roy et al., 2018, WRHA, 2017, Wood, 2016). All of the above should be done following the principle of cleaning from higher to lower surfaces and moving from clean to dirty areas (WRHA, 2017, Roy et al., 2018). Thorough cleaning should ensure that the inanimate operating room environment makes a minimal contribution to the incidence of SSI's (Roy et al., 2018).

At three of the four facilities that were surveyed, namely Facilities A, B and C, daily routine theatre cleaning protocols focused on the cleaning of horizontal surfaces (i.e. tables and floors). This could theoretically lead to the accumulation of bacteria on the remaining surfaces, including but not limited to equipment, switches and walls, only to be re-suspended at a later time, thereby contributing to the total bioaerosol load. In comparison, all surfaces including the walls were cleaned at Facility D with an aerosolized disinfectant being used to target surfaces which may have been missed.

Apart from adequately addressing all surfaces, cleaning cannot be considered thorough unless an appropriate disinfectant is used correctly (i.e. dilution, time and degree of biological material

being determinants of overall efficacy). Of the facilities that took part in the study, three of the four (namely A, B and D) made use of the F10 range of products (a commercial range which combines a quaternary ammonium compound and a biguanide). Despite this range being considered a 'broad spectrum biocide', five bacterial species, namely *Enterococcus faecalis*, *Micrococcus luteus*, *Pasteurella multocida*, *Staphylococcus aureus* and *S. epidermidis* listed as indicator micro-organisms for this product range (F10products, 2015) were all isolated to various degrees at these facilities. Possible explanations for this include the incorrect use of the product (i.e. incorrect dilution rates or application times), inadequate cleaning, reintroduction of organisms into the theatre, resistance development or a combination of these factors.

In contrast to the other establishments, Facility C utilized a high foaming chlorinated detergent instead of F10 to routinely disinfect their surgical theatre. With Gram-positive organisms being deemed more susceptible to quaternary ammonium compounds and biguanides (CFSPH, N.D.), the components of F10, it could possibly help explain, at least in part, why Facility C, the only facility to not use F10, had the highest relative Gram-positive load.

5.3.4 Overall effect of theatre conditions

Overall, with an average deposition rate of 1CFU every 59seconds, Facility B had the highest sampled bacterial load. Based on observations at the practice, this is most likely due to the high level of room occupancy, the high thoroughfare (even when the theatre was not in use) by staff wearing a minimal amount of surgical attire, in combination with the frequent exposure to the external environment through open doors.

Despite the utilization of surgical attire and continuously implemented restricted access to the surgical theatre, Facility A, with a deposition rate of one CFU every 1 minute 18 seconds, was the second most contaminated facility. The cumulative effect of occupancy (as a result of a large number of consecutive procedures), in conjunction with minimal opportunity for organism dilution were the most likely causative factors.

This was followed by Facility D, which at 2 minutes 30 seconds, had a CFU deposition rate that was nearly twice as long as previously mentioned practices. This figure was the average of both HEPA equipped theatres (at 2 minutes 51 seconds) and those without (2 minutes 13 seconds). Though the sample size was small, from the obtained data the HEPA filtration did

serve to decrease the total number of organisms in support of literature. What does however need to be taken into account is the short period of time for which the HVAC system was turned on prior to the start of the procedures. An insufficient time to remove resident organisms, in combination with a large number of occupants most likely contributed to a CFU deposition rate that was still higher than that of Facility C.

With a deposition rate 4.7 times slower than the most contaminated facility, Facility C had the lowest bioaerosol load of the sampled locations. In the absence of other apparent factors, the most likely reason for the low deposition rate was the relatively low utilization of the room and consequent decreased accumulation of organisms.

From the above it is clear that the bacterial bioaerosol load is dynamic in nature and influenced greatly by a multitude of factors.

5.4 Pathogenicity of isolated organisms

5.4.1. Overview

Arguably, the pathogenicity of the isolated organisms may be more important than the deposition rate, particularly when evaluating the data from a surgical site infection risk perspective. When the organisms were evaluated in terms of their pathogenicity, 10 of the 53 isolated bacterial species, comprising a total of 37.2% of the collected sample, have been previously implicated in canine or feline surgical site infections. Represented genera included *Micrococcus* (*Micrococcus luteus*, *Micrococcus* species), *Pseudomonas, Streptococcus, Enterococcus, Enterobacter* and *Staphylococcus* (*S. pseudintermedius, S.aureus*, COPS, CONS). This is represented graphically in figure 5-1 below. Discussions on the most important follow thereafter.

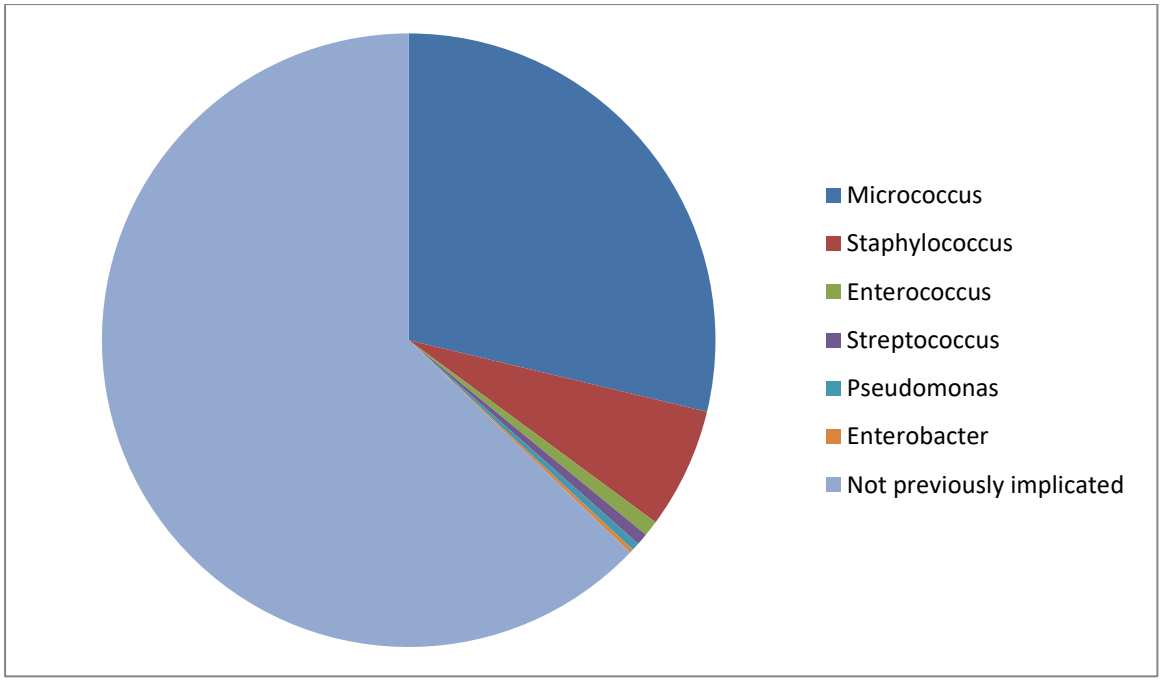

Figure 5-1 Genera previously implicated in canine and feline SSI's represented as a percentage of total isolates identified in this study.

- ***Staphylococcus***: Considering that these organisms are normal canine skin commensals commonly isolated from the nares, mouth, anus, groin and forehead (Schmidt et al., 2014), the presence thereof in a small animal veterinary practice is not unexpected. Staphylococci have become the organisms most frequently implicated in surgical site infections in both the human and veterinary fields (Verwilghen and Singh, 2015, Mellinghoff et al., 2018) and therefore, the isolation thereof is still concerning. In a study evaluating SSI rates in all canine surgical procedure types, performed over a ten month period in Ontario, Turk et al. (2015) demonstrated that 74% of the SSI's were caused by staphylococci. In a Swedish study, which gathered samples from 7 veterinary facilities over a three year period, Windahl et al. (2015) found that staphylococci accounted for approximately two thirds of all SSI's. Of these infections, *S. pseudintermedius* accounted for 46% of all isolates (Windahl et al., 2015).

  In our study, *S. pseudintermedius,* which is the species most commonly implicated in canine SSI's (Verwilghen and Singh, 2015, Pompilio et al., 2015), was isolated from all sampled veterinary establishments except Facility D. The isolation of *S. aureus,* the human equivalent of *Staphylococcus pseudintermedius,* at Facility B should

additionally be noted. Though rarer than its canine commensal counterpart, it has also been implicated in small animal surgical site infections (Windahl et al., 2015, Kalhoro et al., 2019). A single isolate from Facility B could not be identified any more specifically than coagulase-positive staphylococci. Considering that both *S. pseudintermedius* and *S. aureus* can be classified as such, it is assumed that the isolate is pathogenic in nature.

Nineteen isolates of staphylococci could not be identified any more specifically than belonging to the coagulase-negative group of staphylococci (CONS). Without further information it is not possible to accurately predict their importance. In veterinary medicine in general however, CONS typically only cause disease in compromised patients, with superficial skin, wound, implant and surgical site infections being the most common manifestations thereof (Weese, 2010, VetBact, 2017). If such an infection occurs, lack of specific identification is not unusual as speciation rarely affects treatment or prognosis and these organisms can thus be treated as a group (Weese, 2010)

- ***Enterococcus***: *Enterococcus faecalis*, isolated at Facilities A and C, forms part of the normal intestinal flora in both humans and animals (Fraser et al., 2018). Despite being a normal commensal, these organisms are among the most frequently isolated from all classes of wounds in humans (Chong et al., 2017, Kau et al., 2005). This extends to surgical site infections. According to the 2014 Annual Epidemiological report published by the European Centre for disease Prevention and Control, following *Staphylococcus*, at 15.2%, *Enterococcus* was the most frequently isolated (ECDC, 2016). Though data was not available to rank veterinary pathogens to the same accuracy, enterococci are considered to be important veterinary surgical site pathogens (Verwilghen and Singh, 2015).

- ***Streptococcus***: Is the next most commonly isolated Gram-positive coccus from surgical sites (ECDC, 2016). When there is a breach in the host's defences, these opportunistic organisms are able to invade tissues and cause a variety of diseases (Gaschen, 2008), including but not limited to pneumonia, septicaemia, necrotizing fasciitis, cholangiohepatitis and arthritis in dogs (Lamm et al., 2010). The reported small animal

surgical site infection rates for this genus vary greatly. The studies conducted by Turk et al. (2015) and Windahl et al. (2015) implicated streptococcal organisms in 5.2% and 24% of canine SSI's respectively; while Kalhoro et al. (2019), who looked at various wound types, isolated *Streptococcus pyogenes* from 25 up to 70% of cases depending on the wound type. In addition 40% of post-surgical wounds were culture positive for *Streptococcus* (Kalhoro et al., 2019).

- ***Micrococcus***: Micrococcal organisms are generally considered to be harmless bacteria commonly found on mammalian skin or mucous membranes (Murray, N.D., Pathogen-Regulation-Directorate, 2010) These organisms are so common in fact that a study conducted by Kloos et al. (1974) demonstrated a prevalence rate of 96% on human skin. The ubiquitous nature of these bacteria helps to explain the high isolation rate of 28.8% in this study. Though generally harmless, under the right conditions and particularly if the patient is immunocompromised, these organisms may cause disease (Pathogen-Regulation-Directorate, 2010). In the human medical field, these organisms have been implicated in indwelling catheter and surgical implant related infections (Kogure et al., 2014, Miltiadous and Elisaf, 2011), while wound and surgical site infections have been documented in the veterinary field (Kalhoro et al., 2019). Despite the infrequent reporting of clinical cases, the omnipresent nature of these organisms can result in the development of a surgical site infection under the right conditions in a susceptible patient.

- ***Pseudomonas***: Though some species can be isolated from healthy humans, the primary habitat of the *Pseudomonas* group of organisms would be soil and water (Iglewski, 1996, Todar, N.D.). Considering that the isolates in this study could not be identified any more specifically than belonging to the *Pseudomonas* species, and that there are more than 140 species belonging to this genus (Iglewski, 1996), only *Pseudomonas aeruginosa*, the species most commonly implicated in infections, will be discussed. *Pseudomonas aeruginosa* has become an increasingly important nosocomial pathogen in both the human and veterinary fields (Bernal-Rosas et al., 2015). Some studies have found the human *P. aeruginosa* surgical site infection rate to be as high as 33.3% (Oguntibeju and Rau, 2004) and 27.78% (Hani and Adnan, 2009). These localized infections may progress to bacteraemia and sepsis which is frequently fatal (Iglewski, 1996). The consequences are equally as devastating in the veterinary field. In a study

which performed deep tissue culture and haemoculture on dogs with wounds and sepsis, *P. aeruginosa* was isolated in 26.86% of cases (Arias et al., 2017).

5.4.2. Organisms implicated in human SSI's

In comparison to the 10 species implicated in canine and feline surgical site infections, when the same isolates were evaluated for their impact in the human field it was found that a total of 25 of the 53 species had, to various degrees, been implicated in human SSI's. In addition to the organisms mentioned above, with the exception of *Micrococcus luteus* but not the entire *Micrococcus* genus, the following genera were represented: *Kytococcus, Corynebacterium, Rhodococcus, Bacillus, Chryseobacterium, Comamonas* and *Acinetobacter*. Of these additional species implicated in human SSI's, at least 7 have been shown to cause some form of infection in small animals. Thus, considering the significant overlap between disease causing organisms in humans and animals, the potential exists that these bacteria, though as of yet not documented to cause surgical site infections in dogs and cats (perhaps due to lack or testing or reporting), may do so in the future i.e. that under the right conditions, the pathogens isolated in this study could potentially lead to surgical site infections, even though their presence is yet to be described in animals.

5.5 Prophylactic antimicrobials

As discussed previously, prophylactic antimicrobial therapy, where indicated, can play an important role in preventing the progression from inoculation to clinical infection. Both Facilities A and B made use of Duplocillin® as part of their standard perioperative protocol, whilst no prophylactic antimicrobial therapy was utilized by Facility C.

Duplocillin is comprised of procaine benzylpenicillin and benzathine benzylpenicillin, both of which fall into the penicillin antimicrobial category (MSD Animal Health, 2014). Penicillin's prevent bacterial cell wall formation by interfering with the final step in peptidoglycan synthesis (Brunton et al., 2018). With the majority of the organisms being Gram-positive in this study, the use of penicillin would appear sound. However, as with the use of any antimicrobial drug, one needs to match the pharmacokinetics of the drug with its pharmacodynamics effect.

Procaine benzylpenicillin consists of benzylpenicillin combined with procaine (a local anaesthetic) in equimolar amounts to form a poorly soluble salt. Following intramuscular injection the combination is slowly absorbed and hydrolysed to benzylpenicillin (Wishart et al., 2017), reaching peak plasma concentrations in approximately 1-4 hours after administration which is then maintained for 24 hours (MSD Animal Health, 2014). Benzathine benzylpenicillin on the other hand consists of two benzylpenicillin molecules reacting with diphenylethylene diamine. This weak soluble compound forms a depot formation at the site of injection which is then slowly released and hydrolysed to benzylpenicillin (Gartlan and Reti, 2018) resulting in peak plasma concentrations after 24 hours which is then sustained for up to 4 weeks (MSD Animal Health, 2014).

As discussed earlier, in order to achieve maximum efficacy, the timing of administration should be such that maximum serum concentration is present at first incision and is maintained above the minimum inhibitory concentrations of the target organisms until closure (Gawande et al., 2009). Neither Facility A nor B achieved this goal. The standard protocol at Facility A was to administer Duplocillin immediately post operatively, whilst Facility B did administer the drug preoperatively but only at the time of induction. The route of administration (intramuscularly) coupled with the formulation of the drug would result in peak plasma concentrations being reached at a minimum of 1 hour post administration. Considering an estimated preparation time of less than 15 minutes and an average surgical time of just over 16min, peak plasma concentrations would therefore only be reached sometime in the postoperative phase, making the use of the Duplocillin inappropriate, unless given 1 to 4 hours before the procedure. This is however not the only concern. Best practice guidelines recommend the discontinuation of surgical antimicrobial prophylaxis immediately post operatively or within 24 hours after wound closure (Bratzler et al., 2013; WHO, 2016), a goal that is not being met considering that plasma concentrations may be maintained for up to four weeks with this product.

In addition to the correct timing for use of the product, the susceptibility of organisms needs to be determined. For this study, we did not look at penicillin G susceptibility as the information on drug use was only obtained after the culture results identified the organisms in question. From records available for clinical strains, the resistance of organisms isolated from small animals in South Africa against penicillin G is very high in the species that could potentially result in SSI's: *Staphylococcus* (71.6%); *Pseudomona*s (95.5%); *Micrococcus* (50.0%); *Enterobacter* (96.0%); *Enterococcus* (41.7%) and *Streptococcu*s (43.3%) (Chipangura et al.,

2017). This tends to suggest that the use of Duplocillin is not the ideal drug for small animal pre-surgical use.

5.6 Antimicrobial susceptibility of isolated organisms

Considering that the volume of antimicrobial use is a driver for the development of antimicrobial resistance and that the development of novel antimicrobials has not been able to keep pace with the rapid rise in resistance (Floris et al., 2020), a one health approach, which not only minimizes use, but that concurrently protects important antimicrobials, is imperative. To this effect the World Health Organization published the "Critically Important Antimicrobials for Human Medicine" list which is a "ranking of medically important antimicrobials for risk management of antimicrobial resistance due to non-human use" (WHO, 2019a).

Upon drafting this guideline, the first expert workshop concluded that the amount and pattern of antimicrobial usage in animals had a direct impact on bacterial resistance patterns and consequent exposure of humans thereto. This, in turn, increased the frequency of infections and treatment failures in the human health sector. The consequences thereof were particularly significant when the involved pathogens were no longer susceptible to antimicrobials considered critically important to human health (WHO, 2019a). Subsequently criteria which took into account the common use for antimicrobials, their target pathogens, as well as the ability of exposed organisms to transfer resistant genes, were developed in order to classify antimicrobial classes according to their importance in human medicine (WHO, 2019a).

Of the ten antimicrobials utilized for susceptibility testing in this study, four were classified as highly important, four were considered to be critically important and two formed part of the most critical group namely 'high priority critically important'.

Highly important antimicrobials, are the antimicrobial classes which fulfil one of two criteria namely: 1) No or limited other therapies are available to treat serious bacterial infections in humans or 2) are used to treat zoonotic infections/acquire resistance genes from non-human sources (WHO, 2017). In this study, the two sulphonamides namely sulfisoxazole and trimethoprim sulphonamide, which affect the nuclear material of bacteria (Dowling et al., 2017), formed part of this group (WHO, 2019a). Tetracycline, which inhibits protein synthesis

and the first generation cephalosporin, namely cephalothin which disrupts the bacterial cell wall (Dowling et al., 2017) were additionally characterized into this group (WHO, 2019a). Of the organisms tested, the percentage of resistance within each antimicrobial class was fairly consistent with 15-23% of the tested isolates being resistant. In comparison, a study conducted by Chipangura et al. (2017) which evaluated the antimicrobial usage patterns by small animal veterinarians in South Africa, detected a much higher overall resistance with 34.6%, 50.6% and 45.1% of isolates being resistant to trimethoprim sulphonamide, oxytetracycline and cephalothin respectively.

The critically important antimicrobials are those drugs that meet both of the previously mentioned criteria (WHO, 2019a). In this class, one has the aminoglycosides (kanamycin and gentamicin in this study) which inhibit protein synthesis (Dowling et al., 2017), as well as ampicillin and amoxicillin/ clavulanic acid which form part of the cell wall inhibiting penicillin group of antimicrobials (Dowling et al., 2017) (WHO, 2019a). The individual isolate resistance to aminoglycosides was fairly consistent in this study at 19 and 21%; the same did not however hold true for the penicillins. Amoxicillin/ clavulanic acid resistance was fairly low at 7.8%, whilst the 50% resistance rate detected towards ampicillin is however unlikely to be a true reflection as only 2 isolates were tested for susceptibility to this drug (in comparison to the second lowest of 35). Chipangura et al. (2017) found a 50.9%, 29.2%, 33.4% and 67.0% resistance to kanamycin, gentamicin, amoxicillin/clavulanic acid and ampicillin respectively. When comparing two commonly used antimicrobials in veterinary medicine, all genera of bacteria displayed either an equal or increased level of resistance to cephalothin in comparison to amoxicillin/clavulanic acid. This result tends to suggest that the organisms are more susceptible to amoxicillin/ clavulanic acid, a finding which is reassuring considering that this antimicrobial is ranked as relatively more critical when compared to cephalothin. More work would however be needed to ascertain under South Africa conditions, if an amoxicillin/clavulanic acid antimicrobial should be used as a frontline antimicrobial in the prevention of SSI's.

For an antimicrobial class to be classified as 'high priority critically important', three criteria must be met in addition to those mentioned above (WHO, 2019a). Firstly a large number of people are affected by diseases for which few alternative effective treatments exist, there is a high frequency of use of the antimicrobial in the human medical field and lastly the active ingredient is used for the treatment of diseases in humans in which there is evidence of

transmission of resistant bacteria/genes from non-human sources (WHO, 2017). The quinolone, enrofloxacin, as well as erythromycin, a macrolide, are both categorized into this group (WHO, 2019a). Of the 102 isolates which were tested for enrofloxacin susceptibility, 16% were considered resistant. Though concerning, it is not nearly as alarming as the erythromycin results in which 40% of all isolates were resistant. This is in contrast to the 32.3% and 0.0006% obtained by Chipangura et al. (2017) for enrofloxacin and erythromycin respectively.

At face value, the degree of resistance prevalent in this study appears to be significantly lower than that obtained by Chipangura et al. (2017), boding well for the sampled facilities. It should however be kept in mind that the results of these two studies are not directly comparable despite both being collected in the South African context. Firstly, one needs to consider that antibiograms were only performed on 20.9% of isolates in this study, increasing the likelihood that tested isolates were not representative of the population as a whole. Secondly, despite an overlap of 12 genera, the study conducted by Chipangura et al. (2017) covered an additional 25 not encountered in this study, this may lead to differences in resistance trends when evaluating per antimicrobial. Finally and arguably most importantly, having identified that 91.16% of clinicians first try empirical treatment before performing antimicrobial susceptibility testing, it is highly likely that the data obtained by Chipangura et al. (2017) was from animals that were non responsive to empirical treatment, thereby selecting for samples that trended towards resistance. All in all, considering that the organisms forming part of the bioaerosol load in this study originated from a combination of healthy patients, healthy surgical staff and the environment; the degree of resistance encountered to these highly important antimicrobial classes is concerning.

Because only a small number of samples were tested in this study, the selected isolates may not have been representative of each species as a whole. It consequently was not possible to accurately comment on resistance trends from a species/genus standpoint. Furthermore, because the method of antimicrobial susceptibility testing that was utilized only measured phenotypic resistance, it was not possible to speculate whether the observed resistance was due to inherent, or acquired and thus possibly transferable mechanisms. For these reasons, the abovementioned aspects will not be discussed further.

6. CONCLUSION

This study started the process of evaluating the role that conditions in veterinary theatres had on surgical wound infections, which, as a field, has been well described in human literature. Being a pilot study, one should however not over interpret findings, particularly considering the small sample size in relation to large number of variables between procedures facilitated at different facilities. Nonetheless, the collated data alluded to trends, which, due their similarity with previously published literature, allowed for certain conclusions to be drawn.

One of the major findings of this study was that 61.8% of the isolates were commensal organisms. As previously described, this bioaerosol accumulation of a theatre environment could be reduced through the use of a HVAC system. Considering however that this is not within the financial means of three of the sampled facilities, the implementation of other, more economical control measures, are recommended. Performing the presurgical patient preparation in a separate room, limiting the number and movement of people in the theatre, both during and between procedures, and adherence to appropriate surgical attire that is regularly changed and cleaned, is imperative. This should be combined with a thorough cleaning protocol whereby the theatre is damp dusted prior to the start of the day's procedures, all surfaces that have been in contact with the patient or that are soiled are cleaned between procedures, with terminal disinfection taking place at a minimum at the end of each procedure day or following a prolonged down time of the room. Implementing the above measures should serve to decrease organism introduction and accumulation.

As mentioned earlier, susceptibility testing was limited due to unexpected complexities in evaluating the samples by the laboratory. As for the isolates that were tested, a trend was evident in that the perioperative antimicrobial practices employed by two of the facilities were not aligned with recommended guidelines. In neither case did the timing of administration allow for appropriate intra-operative therapeutic levels; whilst the long acting nature of the formula tended to create an environment that promoted bacterial exposure for a protracted time. This type of use fails to provide any additional decrease in SSI risk, whilst at the same time increasing the selection pressure on both the target and resident organisms. We further need to highlight that it is less than ideal to use perioperative antimicrobials for a procedure which does

not routinely require it, as focus should instead be placed on enhancing environmental cleanliness.

Though this pilot study did provide basic insight in the composition and antimicrobial susceptibility of the bioaerosol load present in a veterinary theatre during routine canine and feline sterilizations, further studies could follow individual patients post operatively, track surgical site infection occurrence and relate this back to potential managerial practices within the facility. Emphasis could additionally be placed on general antimicrobial use practices within each facility to evaluate its relation to resistance trends encountered. Evaluating the genotype of antimicrobial resistance in these facilities can additionally be looked into.

In summary, this study provided a glimpse into the factors that may contribute to the bioaerosol load within a veterinary theatre. The multitude of contributing factors has created a dynamic reservoir of bacteria that, if not carefully managed, can contribute to the incidence of surgical site infections and consequent increase in antimicrobial use. Emphasis should therefore be placed on optimizing environmental management before antimicrobial use is considered.

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APPENDIX A: ETHICS APPROVAL

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**A study to evaluate the risk of air-borne contamination in non-environmentally controlled veterinary theatres**
PROJECT NUMBER	**V049-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. C van der Merwe**

STUDENT NUMBER (where applicable)	**U_12025870**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPESIES/SAMPLES	***Canis familiarus* & *Felis Domesticus***
NUMBER OF ANIMALS	**129 in total**
Approval period to use animals for research/testing purposes	**June 2018 - June 2019**
SUPERVISOR	**Prof. V Naidoo**

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 25 June 2018
CHAIRMAN: UP Animal Ethics Committee	Signature

54285-15

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Research Ethics Committee

PROJECT TITLE	A Study to evaluate the risk of air-borne contamination in non environmentally controlled veterinary theatres
PROJECT NUMBER	REC036-18
RESEARCHER/PRINCIPAL INVESTIGATOR	C van der Merwe

DISSERTATION/THESIS SUBMITTED FOR	MSc

SUPERVISOR	Prof V Naidoo

APPROVED	Date	27 August 2018
CHAIRMAN: UP Research Ethics Committee	Signature	A.M. Duncan