

# Airborne bacteria in veterinary surgical theatres in South Africa

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The bioaerosol composition of the theatre environment plays a determining role in the development of surgical site infections (SSIs). It has been demonstrated that the concentration of viable airborne bacteria is influenced by the level of room occupancy, utilisation of surgical attire and importantly, proper ventilation systems, which are often lacking in the average veterinary facility.

The aim of this study was to evaluate the airborne bacterial load encountered in non-environmentally controlled small animal veterinary theatres during routine surgical sterilisations, and to correlate these findings with the managerial practices at the facility.

Four veterinary facilities with differing throughputs and managerial practices were recruited into the study. Blood agar settle plates, open from first incision to last suture, were used to quantify organisms that could settle in an incision.

The 45 plates yielded 487 bacterial isolates (53 species). The *Micrococcus* (28.8%) and *Staphylococcus* (16.8%) genera were predominant. Of the isolates 61.8% were classified as human/small animal commensals and 37.2% belonged to species previously implicated in small animal SSIs.

Specific trends were additionally evident in the bioaerosol loads. High room occupancy, lack of surgical attire and exposure to the outside environment were associated with higher bacterial counts. Accumulation from consecutive procedures was identified and linked to total occupancy time of the room. Current mitigation measures were not ideal to minimise the SSI risk. Routine, frequent and thorough cleaning in combination with surgical attire utilisation is recommended to reduce the bioburden for patient benefit.

**Keywords:** surgical site infection, non-environmentally controlled, open-air theatre, air-borne bacteria, veterinary theatre, clean procedures

## Introduction

Veterinary medicine is dedicated to the promotion of health and the prevention of disease in animals. The surgical sterilisation of canines and felines achieves this goal 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). Despite the medical benefits from these procedures, the development of disease in the form of surgical site infections (SSIs) fails to meet a key goal in the principle of 'do no harm'.

In its least severe form, SSIs result in delayed wound healing and increased patient morbidity, whilst in other cases leading to protracted hospital stays, secondary complications and even death (Darouiche 2016; Badia et al. 2017; Nelson 2011). 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 (Spohrc et al. 2012; Eugster et al. 2004).

The development of a SSI 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 flora, or exogenously from the surrounding environment (Owens

& Stoessel 2008). In the case of a clean surgical procedure, as would be the case for a routine sterilisation, it has been shown that approximately 98% of the bacterial load is derived from airborne pathogens (Whyte et al. 1982). This is in line with the statement made by Sadrizadeh & 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). By extension, if the degree of airborne contamination can be controlled, the incidence of SSIs can be reduced.

The airborne bacterial 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 (Al-Waked 2010; Roy et al. 2018; Shaw et al. 2018). This correlation between the room occupancy and bacterial load is so strong, that it has been shown that each additional person increases the SSI risk 1.3 fold (Eugster et al. 2004). In order to 'contain' shedding, staff are required to wear appropriate surgical attire which consists of masks, gloves, caps, scrub suits and gowns (Gawande et al. 2009). 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% of these cells being expected to carry microorganisms (Al-Waked 2010).

Table 1: Summary of facility conditions

	Facility A	Facility B	Facility C	Facility D
<b>General Facility Information</b>				
<b>Facility throughput</b>	High throughput $\geq 10$ sterilisations on a daily basis.	Medium throughput $\pm 4-5$ procedures a day. Procedures not necessarily limited to sterilisations.	Low throughput practice, approximately two surgical procedures a day.	Facility only utilised approximately once a month.
<b>Theatre cleaning</b>	Cleaned upon completion of the day's procedures, with tables only being cleaned if wet/dirty. A QAC/biguanide-based product in addition to a sodium hypochlorite product was used.	Floor and tables cleaned twice daily with a QAC/biguanide product. Tables cleaned between procedures.	Cleaned at the end of each day with a high foaming chlorinated detergent. Between procedures the table was cleaned with a chlorhexidine-based product.	The theatres were cleaned with QAC/biguanide before the start of each procedure day. This included cleaning walls, floors and tables. A fogger which aerosolised the same disinfectant was additionally used.
<b>Procedure Protocols</b>				
<b>Patient</b>				
<b>Preparation</b>	Patient pre-surgical scrub performed in a separate room.	Patient pre-surgical scrub performed in a separate room.	Patient preparation performed in the theatre for the majority of procedures.	Patient pre-surgical scrub performed in a separate room.
<b>Draping</b>	Patient partially draped with sterile drapes.	Based on the attending veterinarian's discretion, sterile drapes were utilised to partially drape the patient in just over half of procedures, while no drapes were utilised for the remaining procedures.	Clean but non-sterile drapes used to partially cover patient.	Patient draped fully with sterile drapes.
<b>Anti-microbial use</b>	Procaine benzylpenicillin/benzathine benzylpenicillin formulation given IM, upon completion of procedure.	Procaine benzylpenicillin/benzathine benzylpenicillin formulation given IM during preparation.	No antimicrobials given.	Terminal surgery – no antibiotics given.
<b>Surgical Personnel/ Attire</b>				
<b>Number of surgical personnel present</b>	Never more than two.	Varied between three and six.	Varied between two and four.	D1: Seven to eight surgical personnel were present the majority of the time D2: Varied between five and eight.
<b>Gloves</b>	Non-surgical gloves utilised.	Surgical gloves utilised.	No surgical gloves utilised.	Non-surgical gloves.
<b>Face mask</b>	Only worn by the surgeon.	Varied greatly – from all staff members wearing masks to no members wearing masks, with variations in between.	Masks never utilised.	D1: All personnel wore masks. D2: Majority of personnel wore masks.
<b>Scrub Cap</b>	Surgeon only.	No members of staff.	No members of staff.	All personnel.
<b>Surgical gown</b>	Not utilised.	Not utilised.	Not utilised.	All personnel.
<b>Air Conditions</b>				
<b>Doors/ Entrances</b>	One. Remained closed for the majority of the time.	Three 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 three being open during two procedures.	Three inside doors lead into the theatre. Doors were kept closed for the majority of procedures, with one door being left open on occasion.	Two inside doors in each of the theatre's, one of which was an inter-leading door. All doors remained open.
<b>Movement of people</b>	Minimal movement.	Intermediate to high movement.	Minimal movement.	High throughput with a lot of movement.
<b>HVAC systems</b>	None.	Air conditioner which was on for 16% of procedures.	Air conditioner which was on for all procedures.	D1: HEPA filtered. D2: No filtration unit.

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

**Key:** D1: HEPA filtered theatres at facility D, D2: non-HEPA filtered theatres at facility D, IM-Intramuscular, HEPA – High efficiency particulate air, HVAC: Heating ventilation and cooling, QAC: Quaternary ammonium compound

To further mitigate the introduction of bacteria, theatres can be equipped with heating ventilation and air conditioning (HVAC) systems. These units not only serve to exchange the entire volume of air in the operating room on a regular basis, but through the utilisation of high efficiency particulate air (HEPA) filters, remove particles larger than 0.3 µm with an efficiency of 99.97% (Mangram et al. 1999). Despite the value of HVACs, most veterinary facilities do not have the financial resources available for an appropriate ventilation system. These less than ideal conditions could theoretically lead to veterinary theatres being contaminated with high bacterial loads. This can in turn increase the risk of SSIs and thereby the utilisation of otherwise unnecessary prophylactic antimicrobials as a compensatory measure. To investigate if this is the case in South Africa, this study evaluated the airborne bacterial load encountered in non-environmentally controlled veterinary theatres during routine canine and feline sterilisation. The secondary objectives were to identify isolated organisms to ascertain their potential for surgical site infections, and their antimicrobial susceptibility.

### Material and methods

Four veterinary facilities were evaluated in the study. Included were three first opinion, small animal veterinary practices without ventilation systems, which were considered to have high, intermediate and low surgical caseloads. The Biomedical Research Centre (Onderstepoort, University of Pretoria, Facility D), made up of four theatres, served as a control facility. Because only a limited amount of data was collected at Facility D, theatres 1 and 2, which were essentially identical and contained HEPA filtration systems, were treated as a single unit – namely D1. Whilst the non HEPA filtered theatres, 3 and 4, formed D2.

The details of the various theatres are reported in Table I. The study was approved by the Animal Ethics Committee of the University of Pretoria (V049-18) and the Research Ethics Committee of the University of Pretoria (REC036-18).

### Sample collection

Samples were taken during routine canine ovariohysterectomies and orchidectomies, as well as during feline ovariohysterectomies. No procedures were booked specifically for the purpose of this study. Sampling was through the use of settle plates as described by Tršan et al. (2019), on blood agar prepared specifically for this study. Settle plates (one per procedure) were placed at the same height as the patient and as close as possible to the incision site (maximum of one metre), without affecting the sterile field. Plates were placed on the lateral aspect on 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. The samples reached the laboratory for further processing within four hours of collection. Facility D was sampled once during a training workshop. To simulate the average duration of a surgical sterilisation, two plates, placed in each of the four theatres, were opened upon first incision and closed after 20 minutes. The Department of Veterinary Tropical Diseases bacteriology laboratory and the Potchefstroom Veterinary Laboratory assisted with the processing of these samples.

### Bacterial identification

Plates were incubated at  $37 \pm 2$  °C for 48 hours, at which point the number of colony-forming units were manually counted. Pure cultures were subject to primary identification, with the Sensititre ARIS 2x automated bacterial identification system being used to identify organisms to the genus or species level. Bacterial identification was done for all collected samples. Once identified, organisms were grouped into Gram-positive and Gram-negative. The natural habitat of each of the sampled species was researched, allowing isolates to be further subcategorised into commensals (listed as forming part of the normal microflora of humans/canines and felines), and non-commensals. Organisms which are occasionally isolated as commensals, but commonly found in the environment, were categorised as non-commensals. Resources used to classify these organisms are listed in Appendix A.

### Antimicrobial susceptibility testing

Susceptibility testing was undertaken with appropriate antibiotic discs, including kanamycin (30 µg), 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). Antimicrobials were selected based on the guidelines set out by the Clinical and Laboratory Standard Institute (CLSI). The quality control of the test was assured using an individual ATCC strain for each organism tested. Result interpretation was based on the definitions provided by this standard. Organisms are classified as susceptible when the zone diameter is at or above the susceptible breakpoint, i.e. is at or above the drug level that can be achieved at the site of infection when used at recommended dosages. The intermediate category implies reduced efficacy when compared to susceptible organisms. A resistant isolate on the other hand would not be inhibited by usually attainable drug concentrations at the site of infection due to a zone diameter below the susceptible breakpoint or specific antimicrobial resistance mechanisms (CLSI 2020). Multidrug resistance was based on the definition utilised by Siegel et al. (2007) whereby organisms were considered to be multidrug resistant when resistance to two or more antimicrobial classes was demonstrated.

### 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 (CFU) 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 summarised by the equation:

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

The bioaerosol load, being the colony forming units per volume of air (cfu/m<sup>3</sup>) was calculated using the Omeliansky formula:

$$N = 5a \times 10^4 (bt)^{-1}$$

whereby  $N$  = colony forming unit per cubic meter of air ( $\text{cfu}/\text{m}^3$ ),  $a$  = number of colonies per settle plate,  $b$  = surface area of settle plate in  $\text{cm}^2$ , and  $t$  = time exposure (minutes) (Najotra et al. 2017).

## Results

### Settle plate results

The total number of procedures and total sampling times were 12 (209 min), 12 (196 min) and 13 (278 min) for facilities A to C. Facility D was sampled for 160 minutes, representing two plates in each of the four theatres for 20 min. Overall the 45 settle plates, covering 843 minutes, yielded 487 bacterial isolates (53 species) with no plate being negative on culture. Average deposition rates (time/CFU) were 1 min 18 sec, 59 sec, 4 min 36 sec and 2 min 30 sec for facilities A-D respectively; whilst mean air contamination ( $\text{cfu}/\text{m}^3$ ) was calculated to be  $691 \pm 357$ ,  $788 \pm 338$ ,  $153 \pm 109$  and  $314 \pm 94$  for these same facilities. An overview of the results obtained is available in Table II. A more detailed breakdown of the mean air contamination is available in Figure 1 thereafter.

Each dot represents a single settle plate, whereas the red crosses represent the mean microbial air contamination for each facility. The broken line at  $180 \text{ cfu}/\text{m}^3$  represents the maximum acceptable standard of air contamination in a working theatre as set by the Healthcare Infection Society (Stauning et al. 2018; Hoffman et al. 2002). Dots above this line are above the maximum recommended levels.

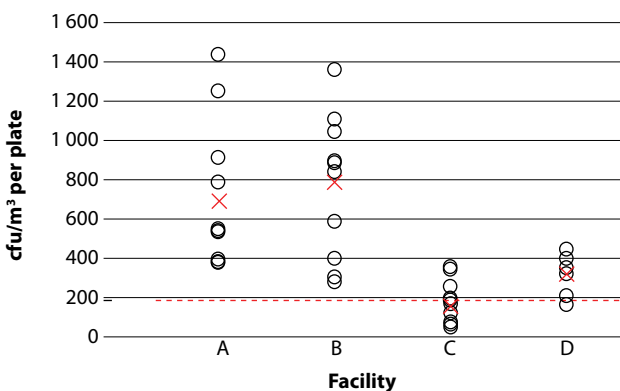


Figure 1: Microbial air contamination

Table II: Summary of data obtained at each facility

	Facility				
	Overall	A	B	C	D
<b>Plates</b>	45	12	12	13	8
<b>Total Collection time (min)</b>	843	209	196	278	160
<b>Deposition Rate (Time/ CFU)</b>	1 min 44 sec	1 min 18 sec	0 min 59 sec	4 min 36 sec	2 min 30 sec
<b>Species</b>					
<b>Total</b>	53	33	36	17	22
<b>Commensal (%)</b>	45.3	48.5	44.4	52.9	36.4
<b>Isolates</b>					
<b>Total</b>	487	160	201	62	64
<b>Commensal (%)</b>	61.8	63.1	59.7	74.2	53.1
<b>Total occupancy time (min)</b>	1986	408	929	766	720
<b>Bioload per person</b>	0.15	0.25	0.13	0.06	0.05

Key: CFU – Colony forming unit

### Classification of the organisms

Across all facilities, Gram-positive isolates were significantly more abundant, with the trends in the predominant genera being evident between facilities. A detailed breakdown is presented in Table III.

When the isolates were categorised into commensals (i.e. those that form part of the normal microflora of humans/small animals) and non-commensals based on published literature, commensals accounted for 53.1% of the isolates at facility D (50.0% in the HEPA-equipped theatres and 55.6% in the non HEPA-equipped theatres); 59.7% at facility B; 63.1% at facility A and 74.2% at facility C. This can be visualised in Figure 2.

For the commensal bioload per person per minute, facility D was the lowest at 0.05 commensals per person per minute, with facility C, B and A at 1.2x, 2.6x and 5.0x that of facility D. When the isolates are evaluated in terms of potential pathogenicity, 10 species that have previously been implicated in small animal surgical site infections were identified. Implicated species included *Micrococcus luteus*, *Micrococcus* species, *Pseudomonas* species, *Streptococcus* species, *Enterococcus faecalis*, *Enterobacter cloacae*, *Staphylococcus pseudintermedius*, *S. aureus*, coagulase-positive staphylococci (COPS) and coagulase-negative staphylococci (CONS). Despite forming only 37.2% of the total

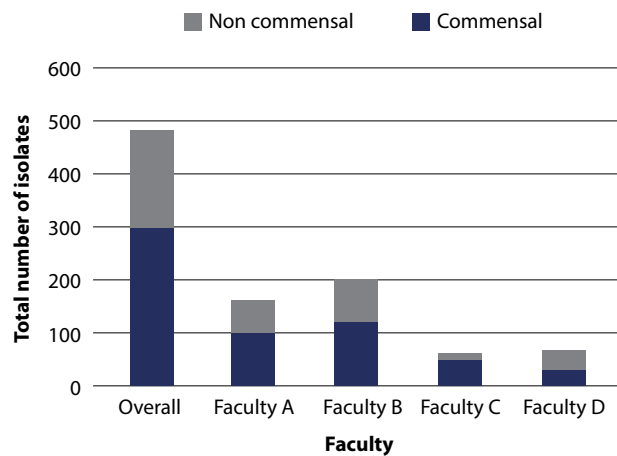


Figure 2: Comparison of the number of commensal vs. non-commensal isolates collected at each facility

Table III: Isolated genera as a percentage of facility total

Genus	Percentage of facility total (%)				
	Overall (n = 487)	Facility A (n = 160)	Facility B (n = 201)	Facility C (n = 62)	Facility D (n = 64)
<b>Gram-positive</b>	<b>81.31</b>	<b>77.50</b>	<b>80.60</b>	<b>87.10</b>	<b>84.38</b>
<i>Micrococcus</i>	28.75	33.75	23.38	35.48	26.56
<i>Staphylococcus</i>	16.8	13.75	19.4	20.97	12.50
<i>Corynebacterium</i>	11.70	3.75	20.40	8.06	7.81
<i>Bacillus</i>	8.62	15.00	5.97	4.84	4.69
<i>Streptococcus</i>	6.98	4.38	5.97	11.29	12.50
<i>Kocuria</i>	2.46	2.50	0.50	1.61	9.38
<i>Trueperella</i>	2.26	1.88	2.49	3.23	1.56
<i>Rhodococcus</i>	1.64	0.00	2.49	0.00	4.69
<i>Enterococcus</i>	1.03	2.50	0.00	1.61	0.00
<i>Aerococcus</i>	0.62	0.00	0.00	0.00	4.69
<i>Kytococcus</i>	0.41	1.25	0.00	0.00	0.00
<b>Gram-negative</b>	<b>18.69</b>	<b>21.25</b>	<b>19.40</b>	<b>12.90</b>	<b>15.63</b>
<i>Moraxella</i>	4.72	2.50	8.46	1.61	1.56
<i>Chryseobacterium</i>	3.70	3.75	2.99	3.23	6.25
<i>Acinetobacter</i>	2.46	4.38	2.49	0.00	0.00
<i>Yersinia</i>	1.85	3.75	1.00	1.61	0.00
<i>Sphingomonas</i>	1.23	0.63	1.00	1.61	3.13
<i>Comamonas</i>	0.82	1.25	1.00	0.00	0.00
<i>Riemerella</i>	0.62	1.25	0.00	0.00	1.56
<i>Pasteurella</i>	0.62	0.63	0.00	3.23	0.00
<i>Chryseomonas</i>	0.00	0.00	0.50	0.00	0.00
<i>Pseudomonas</i>	0.62	1.25	0.00	0.00	0.00
<i>Elizabethkingia</i>	0.41	0.00	0.50	0.00	1.56
<i>Empedobacter</i>	0.41	0.63	0.50	0.00	0.00
<i>Enterobacter</i>	0.41	0.00	0.50	0.00	1.56
<i>Psychrobacter</i>	0.41	0.00	0.50	1.61	0.00
<i>Pantoea</i>	0.21	0.63	0.00	0.00	0.00
<i>Vibrio</i>	0.21	0.63	0.00	0.00	0.00

Table IV: Antimicrobial resistance trends grouped according to genus

Genus	Number of tested isolates that showed resistance to each antimicrobial											Total resistance per genus <sup>a</sup>		Multidrug resistance <sup>b</sup>	
	Isolates tested	Kanamycin	Amoxicillin/ clavulanic acid	Cephalothin	Enrofloxacin	Sulfisoxazole	Trimethoprim/ sulphamethoxazole	Erythromycin	Tetracycline	Gentamycin	Ampicillin	n	%	n	%
	n														
<i>Enterobacter</i>	1	NT	1	1	1	1	NT	NT	1	1	NT	1	100.0	1	100.0
<i>Enterococcus</i>	2	2	0	1	2	2	NT	1	2	NT	1	2	100.0	2	100.0
<i>Pseudomonas</i>	1	0	0	1	0	0	NT	NT	0	NT	NT	1	100.0	0	0.0
<i>Staphylococcus</i>	25	1	0	0	1	NT	5	11	8	NT	NT	18	72.0	6	24.0
<i>Streptococcus</i>	8	7	0	0	3	NT	20	1	3	NT	NT	7	87.5	4	50.0

NT indicates not tested for that specific antimicrobial.

<sup>a</sup>Total resistance per genus indicates the number of isolates which were resistant to at least one antimicrobial.

<sup>b</sup>Organisms in genus that were resistant to two or more antimicrobial classes.



isolates, at least one pathogenic organism was isolated in 88.9% of procedures.

### Antimicrobial susceptibility

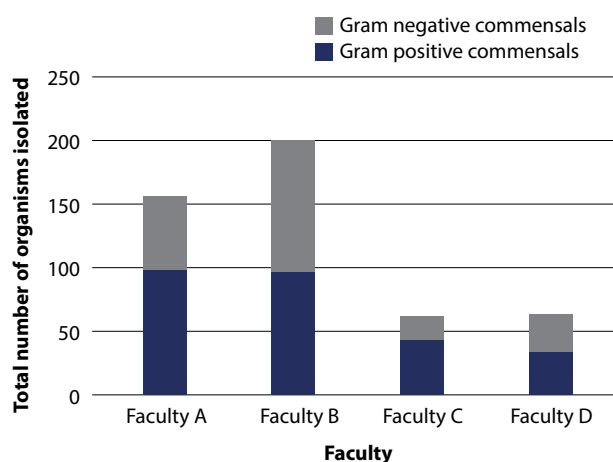
Though antimicrobial susceptibility testing was performed, overall, due to sample loss (i.e. pure cultures which did not survive cold storage for further antimicrobial susceptibility testing), too few samples were available to obtain statistically valid results. To illustrate the potential for air-borne bacteria to carry resistance, the pooled results of some of the more commonly encountered genera are presented in Table IV.

### Discussion

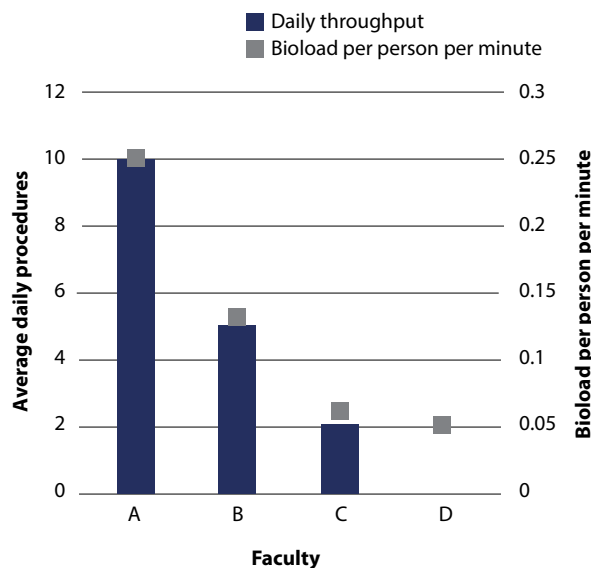
As expected, a diverse group of bacterial organisms were isolated during the course of this study. Of the isolates, 61.8% could be classified as human and/or companion animal commensals, while 37.2% belonged to species that have previously been implicated in small animal SSIs.

Considering that a single patient was present in each room at the time of sampling and that these patients were draped (albeit drapes may have been applied slightly differently), one can expect the level of shedding from the animals between procedures and facilities to be similarly low, thereby making the person(s) in theatre and their level of surgical attire, the major contributor to the bioload.

As a way of comparing the effect that personnel had on the bacterial bioload between facilities, the commensal deposition rate per total occupancy time (i.e. the bioload per person per time period) was calculated. Based purely on the degree of utilisation of surgical attire, it would be expected that facility D would have the lowest commensal bacterial load, followed by facility A and then B, with facility C, due to its relative lack of protective clothing, having the highest commensal bioload per person. The calculated result did however not follow the expected trend, instead, at  $A > B > C > D$ , it was nearly the opposite. A likely explanation would be the extended survival of organisms in the environment and the consequent cumulative effect that room occupancy and consecutive procedures had on bacterial counts.



**Figure 3:** Classification of commensal isolates into Gram-positive and Gram-negative



**Figure 4:** Relationship between facility throughput and bioload per person

Of the 24 species that were classified as commensals, all but four were Gram-positive. Gram-positive organisms, due to their relatively thick peptidoglycan layer of highly cross-linked chains (Salton & Kwang-Shin 1996), have a superior ability to survive adverse environmental conditions (Tolabi et al. 2019). *Staphylococcus epidermidis*, which was isolated at three of the facilities for example, has been shown to remain viable in the environment for five days (Thompson et al. 2011).

When re-evaluating the commensal bioload per person per time period with this knowledge in mind, a trend emerges. Due to the high procedure load, the cumulative effect of theatre occupancy at facility A would be quite high, explaining the high bacterial load despite the relatively good level of surgical attire utilisation. This is in direct contrast to the low throughput facility C which yielded a low bioload per person despite almost no surgical attire being worn. This leads to the conclusion that, in the veterinary surgical theatres evaluated, the level of contamination is linked to the total amount of time persons spend in the theatre, a trend which is evident in Figure 4.

Facility D was noted as having a daily throughput of '0' as it is only used approximately once per month.

In a theatre environment with a HVAC system which replaces the volume of air, this bioload would be reduced by the air system before organisms have the chance to settle into the wound site. Since theatres often do not have installed ventilation systems, the one area of debate focuses on opening windows and doors to allow for a degree of fresh air-flow as a way of diluting some of the already present commensal organisms. Considering however that "outdoor air is thought to be the most important source of indoor micro flora" (Lina et al. 2019), this may not necessarily be true. The current study offers some insight into this.

At facility A, the theatre door and windows remained closed whether or not the room was in use; while facility B, in general, had one door and at times three doors left open with large amounts of movement. Though dilution was evident at facility B, as it had the lowest proportion of commensal organisms, overall

it had the highest plate count, higher even than facility A which recorded double the number of procedures on a daily basis. The introduction of contaminated outside air is therefore likely a major contributing factor. In order to find the balance between a completely closed system, where no dilution takes place, and seeding the environment with additional pathogens through contaminated outside air, it is recommended that veterinary theatres without adequate mechanical ventilation systems allow fresh air introduction for a few hours following completion of the procedures for the day, as seen in facility C. Thereafter, proper mechanical cleaning should take place before the theatre is used again, whilst other mitigating measures such as performing patient preparation outside of the theatre, correctly draping the patient once in the operating room (OR) and utilising appropriate surgical attire (gloves, masks, caps and gowns) should be used to decrease initial seeding.

In order to be truly effective, theatre cleaning should not be thought of as a singular event, but rather a continuously implemented process. All horizontal surfaces should be damp dusted at the start of each day, spills and biological waste should be taken care of intraoperatively. All surfaces and equipment in the immediate vicinity of the operative area or that have been in contact, either directly or indirectly, with the patient or staff, should be cleaned between procedures. 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). Apart from adequately addressing all surfaces, cleaning cannot be considered thorough unless an appropriate disinfectant is used correctly, with dilution, time and degree of biological material being determinants of overall efficacy.

Overall the combination of daily throughput, theatre occupancy, surgical attire utilisation, fresh air introduction and cleaning protocols resulted in a mean air contamination 1.7, 3.8 and 4.4 times higher at facilities D, A and B than the 180 cfu/m<sup>3</sup> maximum recommended by the Healthcare Infection Society (EAI4). The higher than expected levels in Facility D was associated with the HVAC only being used intermittently and not constantly as one would expect. Facility C, at 153 cfu/m<sup>3</sup>, was the only facility where the average bioaerosol load was within the standards for a working theatre; though some procedures did exceed the recommended limit. One of the concerns with having high circulating levels of bacteria in a theatre environment is their potential ability to be infectious. Considering that counts above 700 cfu/m<sup>3</sup> have been associated with a significant risk of airborne infection in the human medical field (Parker 1978), that 37.2% of isolates belonged to species that have previously been implicated in canine or feline surgical site infections, and that at least one of these pathogens was isolated in 88.9% of procedures, the results for this study are concerning.

Though, due to various technical reasons, only a small number of isolates were evaluated for their antimicrobial susceptibility, resistance was clearly evident in the sampled population.

Considering that these organisms would not only require more extensive treatment should a surgical site infection develop, but that they could harbour and potentially disseminate antimicrobial resistance genes, concern is not unjustified. It should additionally be noted that the CLSI standard only included information for 23 of the 41 species that were tested for their antimicrobial susceptibility. Since the zone of inhibition or the minimum inhibitory concentration is based on the interaction between the pharmacodynamics and pharmacokinetics of a specific drug in the species of interest, airborne infections from these other organisms may be more difficult to treat than the common veterinary pathogenic bacteria.

Another concern evident in this study was the manner of use of perioperative antimicrobials as a way of mitigating SSI risk. When employed correctly, prophylactic antimicrobial therapy ensures that adequate plasma concentrations are reached prior to the first incision, suppressing bacterial multiplication to the extent that the host defence mechanisms can prevent the progression to infection (WHO 2016; Verwilghen & 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 later than two hours prior to the start of the procedure (WHO 2016), with the exact pre-surgical dosing interval being dependant on the antimicrobial agent's half-life (WHO 2016). Two facilities made use of a procaine benzylpenicillin and benzathine benzylpenicillin (long-acting) formulation as part of their standard perioperative protocol. From published pharmacokinetic information for this particular long-acting formulation, penicillin concentration only peaks one to four hours after the recommended intramuscular administration (MSD-Animal-Health 2014). Administering the formulation either directly prior to the start of the procedure or immediately postoperatively, as evident in this study, is unlikely to be effective. The long-acting benzyl-penicillin portion of the formulation, which may last up to four weeks, furthermore does not comply with current best use guidelines which conclude that prolonging surgical antimicrobial prophylaxis beyond doses given intraoperatively, does not further decrease the SSI rate (WHO 2016). Overall, considering that it is currently recommended that short (< 90 minute), clean, non-orthopaedic procedures carried out on veterinary patients classified as ASA 1 or 2 (i.e. low risk) do not require antimicrobial prophylaxis (Spohrc et al. 2012; Nelson 2011), emphasis should be placed on correct environmental management before antibiotics are employed.

## Conclusion

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, could contribute to the incidence of SSI and consequent increase in antibiotic use. Emphasis should therefore be placed on optimising environmental management before antibiotic use is considered.

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### Conflict of interest

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this paper.

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### Ethics

Ethical approval was obtained from the Faculty of Veterinary Science Research Ethics Committee (V049-18) as well as from the University's centralised Animal Ethics Committee (REC036-18).

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### Appendix A - List of references used to determine the commensal status of isolated organisms

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