A study of the transmission pathways of organisms associated with nosocomial infections at a veterinary academic hospital

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Declaration

I, Dikeledi Carol Sebola declare that the thesis, which I hereby submit for the degree DOCTOR OF PHILOSOPHY in Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, is my work and has not previously been submitted at this or any other tertiary institution.

SIGNATURE: …….

DATE: …….

Ethics statement

The author whose name appears on the title page of this thesis has obtained the required ethics approval for the research described in this work.

The author declares that they have observed the ethical standards required in terms of the University's Code of ethics for scholarly activities.

Dedication

This thesis is dedicated to the Sebola family, in particular, my parents Mrs Makoma Sophie Sebola, and my late father Mr Mabusha Sebola for their continuous support in getting this far.

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First and foremost, I give all the glory to the most high GOD for carrying me through this process. It has been a rollercoaster ride and I acknowledge that I wouldn't have been able to do it without God's help.

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Research outputs

Conference Presentations

1. **Dikeledi C. Sebola,** James W. Oguttu, Marleen M. Kock and Daniel N. Qekwana. Hospital-acquired and zoonotic bacterial organisms and their associated antimicrobial-susceptibility profile in veterinary hospitals: A Systematic Review. 23 August 2023 SASVEPM 2023 (ORAL).
2. **Dikeledi C. Sebola,** James W. Oguttu, Mogaugedi Malahlela, Marleen. Kock, Daniel N. Qekwana. Occurrence and characterization of ESKAPE pathogens on the hands of healthcare workers in the intensive care unit at a veterinary academic hospital in South Africa 16 June 2023 American Society of Microbiology at Microbe 2023 (POSTER).

Publications

1. **Dikeledi C. Sebola**, James W. Oguttu, Marleen M. Kock and Daniel N. Qekwana. Antimicrobial resistance patterns of Acinetobacter baumannii and Klebsiella pneumoniae isolated from dogs presented at a veterinary academic hospital in South Africa. Veterinary World. 2023.
2. **Dikeledi C. Sebola**, James W. Oguttu, Marleen M. Kock and Daniel N. Qekwana. Hospital-acquired and zoonotic bacteria from a veterinary hospital and their associated antimicrobial-susceptibility profiles: A systematic review. Frontier for. Veterinary. Science. 2023.

Manuscripts under review and to be submitted

1. **Dikeledi C. Sebola,** James W. Oguttu, Mogaugedi Malahlela, Marleen. Kock, Daniel N. Qekwana. Occurrence and characterization of ESKAPE pathogens on the hands of healthcare workers in the intensive care unit at a veterinary academic hospital in South Africa.

2. **Dikeledi C. Sebola,** James W. Oguttu, Marleen M. Kock, Daniel N. Qekwana. Knowledge and perception of veterinary students on the transmission of hospital-acquired infections and zoonotic diseases at the Veterinary Academic Hospital.

Abstract

Background: Hospital-acquired infections (HAIs) are a major concern in human and veterinary medicine. They are caused by bacterial organisms mainly from the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*). Within the veterinary settings, this group of organisms is often zoonotic and tends to acquire resistance determinants. As a result, most of these bacteria are multidrug-resistant, which limits treatment options and patient prognosis. Organisms associated with HAIs are transmitted mainly through the hands of healthcare workers (HCWs), making hand hygiene the most effective measure to prevent and control infections in healthcare facilities. However, low compliance to hand hygiene has been reported in both human and veterinary hospitals, which increases the risk of transmission of HAIs and zoonotic organisms. To reduce the risk of transmission, a multimodal approach has been recommended. As such, this study aims to use a multimodal approach to assess the pre-intervention required to reduce the transmission of organisms associated with HAIs and zoonotic diseases at a veterinary academic hospital.

Methods: In order to achieve the objectives of this study, a systematic literature review using the PRISMA method was undertaken to describe the organisms responsible for HAIs and zoonotic infections. In addition, antimicrobial resistance genes associated with these organisms were also described. Since the results of the systematic literature review showed there was limited information on the burden of ESKAPE pathogens in South Africa, data on dog clinical cases presented at the veterinary academic hospital between 2007 and 2013 were reviewed. *Klebsiella pneumoniae* and *A. baumannii* isolates were assessed in terms of their burden and antimicrobial resistance patterns. Hands of healthcare workers were also assessed for the presence of organisms associated with HAIs and zoonotic diseases using the polymerase chain reaction (PCR). In addition, each isolate was subjected

to antimicrobial sensitivity testing following the Kirby-Bauer disk diffusion method. In order to assess the level of knowledge of veterinary students regarding the transmission of HAIs, a questionnaire survey was performed assessing the knowledge of students on infection prevention and control (IPC) and the transmission of organisms associated with HAIs.

Results: Bacterial organisms associated with HAIs and zoonosis were reported from clinical cases, environmental surfaces, and items used during patient treatment and care. *Staphylococcus* species was the most reported organism, and some isolates seem to share similar clonal lineage to those reported in humans. In terms of resistant genes, the *mec*A gene was identified in both Methicillin- resistant *Staphylococcus aureus* (MRSA) and Methicillin- resistant *Staphylococcus pseudintermedius* (MRSP), the *bla*CMY-2 gene in *E. coli* and *Salmonella* spp., *flo* genes in *E. coli,* and the *van*A gene in *E. faecium* isolates. *Acinetobacter baumannii* (n=20) and *K. pneumoniae* (n=56) isolates were isolated from bronchoalveolar lavage, foreign objects, bone, urine, skin, blood, ear, nasal, and oral cavity. Sixty percent (60%) of *A. baumannii* were multidrug-resistant (MDR) while 98% were MDR *K. pneumoniae*. Of the students tested (62), at least one of the ESKAPE pathogens were isolated from their hands. *Escherichia coli* was the most isolated (76%, 47/62), followed by *E. faecium* (52%, 22/62), *P. aeruginosa* (48%, 30/62*). A. baumannii* (47%, 29/62), *K. pneumoniae* (27%, 17/62), and *S. aureus* (24%, 15/62). Resistance to at least one antibiotic was high among *E. coli* isolates (100%, 9/9), followed by *E. faecium* (67%, 4/6), *P. aeruginosa* (100%, 13/13), *A. baumannii* (57%, 4/7), *K. pneumoniae* (100%, 7/7), and *S. aureus* (67%, 2/3). Only *E*. coli (42%, 5/12), *E. faecium* (40%, 2/5), *P. aeruginosa* (100%, 13/13)*, and S. aureus* (33%, 1/3) were multidrug resistant. Of the 147 students interviewed most were female (69%, 102/147) followed by male (29%, 43/147). Two (1%, 2/147) students did not indicate their sex. Less than half (41%, 60/147) of the respondents indicated they heard about IPC practices. However, they were aware that jewellery, stethoscopes, ward telephones, and leashes are possible sources of pathogens associated with HAIs.

Conclusion: Bacterial organisms associated with hospital-acquired and zoonotic diseases were reported from clinical cases, environmental surfaces, and items used in veterinary service. The hospital environment where there is human contact had the highest burden of organisms associated with HAIs. Moreover, the ESKAPE organisms were identified in the hands of the students working in the ICU. Organisms associated with HAIs in this study were often MDR which is likely to impact patient care and prognosis. In addition, if contaminated, students would likely pass on these pathogens to other persons and animals. The results of this study further support suggestions that human behaviour plays a crucial role in the transmission of HAIs in veterinary hospitals. The study also shows from the survey that students do not have a good understanding of IPC measures and their role in the prevention of HAIs and zoonotic diseases although taught during lectures.

Table of Content

Table of tables

Table of figures

Abbreviations

Abbreviation	Definition
aac(6')-Ib-cr	Acetylating aminoglycoside-(6)-N-acetyltransferase
ABHS	Alcohol-based hand sanitizers
AME	Aminoglycoside modifying enzymes
AMR	Antimicrobial resistance
APEC	Avian pathogenic *Escherichia coli*
Bp	Base pairs
CACS	Companion animal clinical studies
CAI	Community-acquired infection
CA-UTI	Catheter-associated urinary tract infections
CC	Clonal complexes
CLABI	Central line-associated bloodstream infections
CLSI	Clinical and laboratory standards institute
DNA	Deoxyribose nucleic Acid
dNTPs	Deoxynucleotide triphosphates
EEA	European economic area
AME	Enzymatic modification enzymes
erm	Erythromycin ribosomal methylase
ESBL	Extended-spectrum beta-lactamase
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacteriaceae* species.
ET	Endotracheal tubes
EU	European union
GIT	Gastrointestinal tract
GyrA	Gyrase A
HAIs	Hospital-acquired infections
HCWs	Healthcare workers
ICU	Intensive care unit
IDT	Integrated deoxyribose nucleic acid technologies
IPC	Infection prevention and control
KAP	Knowledge attitude and practice
LB agar	Luria Bertani agar
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
mcr-genes	Mobilized colistin resistance genes

MDR	Multidrug
MHA	Mueller Hinton agar
MIC	Minimum inhibitory concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MRSP	Methicillin-resistant *Staphylococci pseudintermedius*
PBP2a	Penicillin-binding protein
PBW	Buffered phosphate water
PCR	Polymerase chain reaction
PFGE	Pulse field gel electrophoresis
PPE	Personal protective equipment
PRISMA	Preferred reporting items for systematic reviews
Qnr	Quinolone resistance gene
RAPD-PCR	Rapid polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SAS	Statistical analysis system
SCC*mec*	*Staphylococcal* cassette chromosome mec
SDGs	Sustainable development goals
SHV-1	sulfhydryl reagent variable-1
Spp.	Species
SPSS	Statistical package for social sciences
16S- RMTases	16S rRNA methyltransferases
STROBE-Vet	Strengthening the reporting of observational studies in epidemiology veterinary
TAE	Tris-acetate-ethylenediamine tetra acetic acid
Taq polymerase	Thermus aquaticus polymerase
UTI	Urinary tract infection
UV	Ultraviolet
VAP	Ventilator-associated pneumonia
VPH	Veterinary public health
VRE	Vancomycin-resistant enterococci
WGS	Whole genome sequencing
WHO	World health organization
WOAH	World organization for animal health
β-lactamases	Beta-lactamase

Chapter 1 Introduction

1.1 Background

Hospital-acquired infections (HAIs) are a growing concern in veterinary and human medicine (1–5). They are defined as infections that are neither present nor incubating at the time of hospitalization (5). *Staphylococcus aureus, S. pseudintermedius, Pseudomonas* species*, Klebsiella* spp.*, Enterococcus* spp.*, Salmonella* spp., and *Escherichia coli* are among the pathogens commonly associated with HAIs in veterinary hospitals (1,6,7). These bacteria are of increasing concern in veterinary medicine as they acquire resistance determinants. In both human and animal studies, these bacteria have been reported to be multidrug resistant (MDR), which impacts patient prognosis (7,8). Examples of such bacteria included Vancomycin-Resistant Enterococci (VRE), multidrug *E. coli*, and Methicillin-Resistant *Staphylococcus aureus* (MRSA) (5,9,10).

Most bacteria responsible for HAIs are commensal to the intestinal tracts of both humans and animals. Those reported in animals have also been shown to be zoonotic and zooanthroponotic (10). This is not surprising as the veterinary setting is an interphase for the transmission of pathogens between humans, animals, and the environment. Therefore, veterinary personnel, students, and patient owners are at an increased risk of infection (7). In developing countries, this is likely to put a financial strain on an already compromised human health system (7). Moreover, there are reports of human cases associated with HAI pathogens known to cause diseases in animals (11). For example, Weese et al (12) reported an outbreak of MRSA skin infection among healthcare workers (HCWs) after contact with hospitalized horses. Similarly, Johnson et al (13) reported similarities between Avian pathogenic *Escherichia coli* (APEC) isolates from humans and animals.

The transmission of HAIs in hospitals can either be directly through contaminated hands of HCWs or animal-to-animal contact (14,15) or indirectly through contact with contaminated hospital environments, equipment, and fomites (16,17). Healthcare workers are at an increased risk of infection through animal bites, scratches or indirectly through contaminated air in the hospital (18). Furthermore, contaminated veterinary personnel can also carry pathogens from facilities to their homes resulting in community-associated infections (17–19).

Clinical symptoms in both humans and animals differ depending on the bacteria involved. In animals, MRSA is associated with wound infections, septic arthritis, and pneumonia (20,21). While *Enterococcus* species are commonly isolated among urinary tract infection (UTI) cases (22). Among the Gram-negatives, *E. coli, P. aeruginosa* and *A. baumannii* have been implicated in clinical conditions such as pyoderma, otitis externa, UTIs (22), pyothorax, upper airway obstruction, bloodstream infections, and wound infections (23). Similarly, MDR *E. coli*, *Enterobacteriaceae and* MRSA have been associated with UTIs, intra-abdominal infections (24), and skin infections in humans(12).

Antibiotic therapy is often required in most HAI cases and penicillins, aminoglycosides, third and fourth-generation cephalosporins, tetracycline, sulfonamides, enrofloxacin, and marbofloxacin are among the most commonly used antibiotics in both humans and animals (25,26). However, there is a high prevalence of antimicrobial resistance among HAI-associated bacteria, which is a major concern for treatment outcomes. For example, the use of vancomycin (27), and carbapenems in the treatment of MDR pathogens (24). These MDR pathogens have been reported in several outbreaks in veterinary hospitals, especially among large animals (12,28–31)

Infection prevention and control (IPC) remains the cornerstone of the prevention of HAIs in human medicine and has slowly been adopted in veterinary medicine (1,5,30,32). These practices are referred to as standard and transmission-based precautions and they are effective in reducing the burden of pathogenic organisms and reliance on antimicrobials (5,33). They have been used in small animal veterinary practices and include hand hygiene, environmental control, sharps management, vaccination for zoonotic infections, patient management, surveillance, and personal protective equipment (1,34,35). If implemented correctly, the risk of infection to patients, animal owners, and veterinary personnel is reduced (36).

The World Health Organization (WHO) recommends the multimodal approach as the first intervention strategy to be implemented for a sustained improvement of IPC (33,37). Multimodal approaches have shown to be effective compared to a single approach in fields such as developing technology (38), adaptability to change in the education sector (39) and adaptability to treatments in health (40). In humans, these approaches have led to an improvement in hand hygiene compliance and reductions in organisms associated with HAIs (41,42). For example, Salama et al (41) in Kuwait

observed a reduction in organisms associated with HAIs and a decrease in incidences of multidrug-resistant bacterial infections due to an improvement in hand hygiene compliance from 43% to 61.4% after an educational campaign. Given this, its adoption in veterinary medicine is likely to yield similar results (43).

Since transmission of most organisms associated with HAIs occurs through the hands of HCWs, effective hand hygiene remains the most effective means to prevent and control infections in healthcare facilities (14,15,41,44,45). However, low levels of compliance have been reported in both human and veterinary hospitals (14,41,46–48). This has been attributed to the lack of hand washing facilities or alcohol-based hand sanitiser dispensers (14,41,46,49). In addition, a lack of knowledge of hand hygiene compliance, attitude towards hand hygiene practices, and awareness of the importance of hand hygiene compliance in reducing transmission of both HAIs and zoonotic infections are among the factors contributing to low hand hygiene compliance (50).

1.2 Justification

Although multimodal approaches have been shown to be effective in human medicine, their use in veterinary medicine is limited (47). A study done in 2019 at the Onderstepoort Academic Hospital showed deficiencies in the implementation of IPC measures including hand hygiene compliance among personnel. In their study, Sebola and colleagues (51) concluded that this low level of compliance is likely to increase the risk of transmission of organisms associated with HAIs in the Intensive Care Unit (ICU). They recommended that a multimodal approach be implemented to improve hand hygiene compliance and reduce the likelihood of transmission of organisms associated with HAIs and zoonosis.

1.3 Aim and objectives

This study aims to assess the pre-intervention IPC principles required to reduce the transmission of bacteria associated with HAIs and zoonotic diseases at a veterinary hospital. The pre-intervention assessments will include the level of knowledge of veterinary students on HAIs, the identification of bacteria associated with HAI and zoonotic diseases from the environment, and the hands of HCWs. The information generated from this study will contribute to the knowledge of the epidemiology of bacteria associated with HAI and zoonosis in veterinary medicine.

1.3.1 Objectives

This study is divided into the following objectives:

1. To describe bacteria associated with HAIs and zoonotic infections and their antimicrobial-resistant patterns in veterinary hospitals using a systematic literature review.
2. To describe the antimicrobial resistance patterns of *K. pneumoniae* and *A. baumannii* from clinical samples of dogs presented to a veterinary academic hospital in South Africa between 2007 and 2013.
3. Investigate the occurrence of bacteria associated with HAIs in the hands of the students working in the ICU.
4. Describing the antimicrobial resistance patterns of the isolated bacteria from students' hands in the ICU.
5. Investigate the Knowledge of students on the transmission of organisms associated with HAIs.

1.4 Benefits

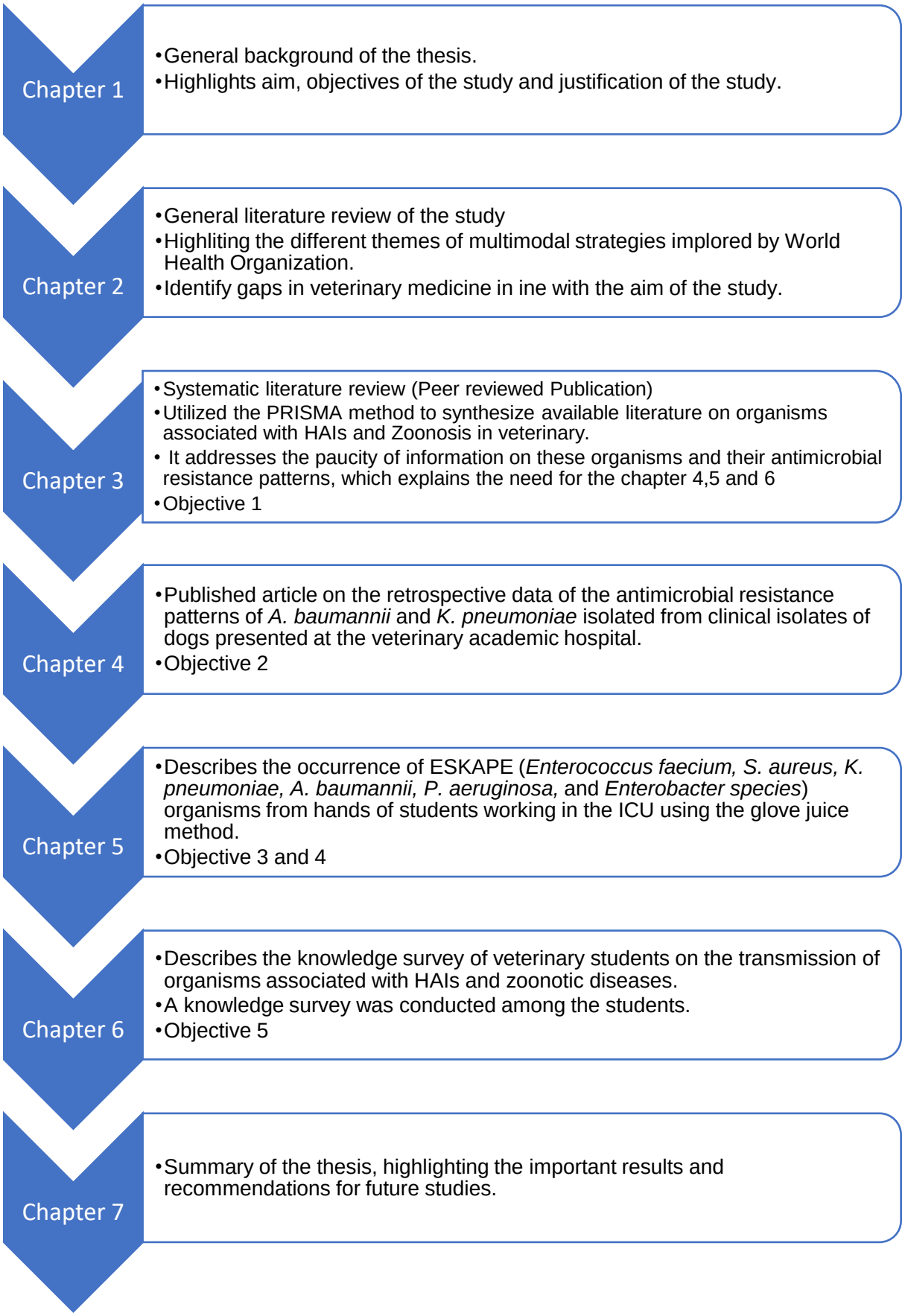
The results of this study will have the following benefits:

1. The results from this study will form a baseline for the surveillance of the organisms associated with HAIs in the veterinary.
2. The results of this study will also be used to guide the veterinary curriculum on infection prevention and control practices.
3. This study will contribute to the national antimicrobial resistance strategic framework, drafted and implemented by the National Department of Health.
4. The study will also contribute to the realization of the Sustainable Development Goals (SDGs) on promoting good health and well-being.

1.5 Structure of the thesis

This thesis is composed of seven chapters. The first chapter presents the general background, aim, and objectives of the study. The second chapter is a literature review of the study, including the different multimodal strategies. Chapters three and four are published articles. Chapter

three consists of the systematic literature review, while Chapter four focuses on the antimicrobial resistance patterns of *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated from clinical isolates of dogs presented at the veterinary academic hospital. Chapter five describes the occurrence of ESKAPE (*Enterococcus faecium*, *S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa,* and *Enterobacter* species) organisms from hands of students working in the ICU. Chapter six describes the knowledge survey of veterinary students on the transmission of organisms associated with HAIs and zoonotic diseases. Finally, chapter seven is a summary of the entire thesis, highlighting the important results and recommendations for future studies (**Figure 1.1)**.



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121 **Figure 1. 1:** This figure summarizes the structure of the thesis and show the main approach used
122 in each chapter.

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Chapter 2 Literature Review

2.1 Background

2.1.1 Infectious diseases and zoonotic infections in veterinary medicine

Infectious diseases in animals have been associated with significant financial losses, high morbidity, suffering, and increased mortality (1,2). Agents that are responsible for infectious disease conditions can be classified into bacterial, viral, fungal, and parasitic (3). *Bovine tuberculosis*, brucellosis, rift valley fever, and foot-and-mouth disease are among the most prevalent infectious diseases affecting livestock, and wild animals (3,4). Whereas in small animal settings rabies, parvovirus infection, babesia, leptospirosis, distemper, and diseases associated with multidrug-resistant bacteria are the most common (5). Furthermore, there is evidence that 60% of emerging infectious diseases in humans may have originated from animals (6,7).

Infectious disease transmission can be direct or indirect, airborne, or vector-borne transmission (5). In veterinary hospitals, transmission can occur as a result of patient-to-patient contact, contact with contaminated environmental surfaces, contaminated fomites, and contaminated hands of healthcare workers and visitors including owners (8–11).

2.1.2 Hospital acquired infections.

Hospital-acquired infections (HAIs) are those infections that patients get after admission into the hospital and infectious agents or toxins that were neither present nor incubating at the time of hospitalization (8,9). Animals are admitted to the hospital for infectious and non-infectious conditions including surgical cases. Most pathogens associated with infectious diseases are involved in either community-acquired infections (CAIs) or HAIs. However, there is evidence to suggest that bacteria associated with HAIs exhibit a high prevalence of resistance to critical, critically important, and important antimicrobials (10–15).

Management of HAIs particularly in the intensive care unit (ICU) remains a significant challenge in both human and veterinary hospitals (8,16–18). However, 30% of these HAIs are

preventable in human and veterinary care facilities. Nonetheless, HAIs are responsible for increased mortality rates, longer hospital stays, increased hospital costs, reduced mobility, and increased antimicrobial drug prescription and costs of treatment in both humans and animals (8,19).

Most HAIs are related to invasive procedures, such as urinary catheters and intravenous catheters, the patient's immune system, and the lack or insufficient implementation of IPC measures (20–22). Hence organisms associated with HAIs have been reported in bloodstream infections, urinary tract infections, and ventilator-associated pneumonia in human and animal studies (8,16,23,24).

Most organisms associated with HAIs are bacterial (22,25) and include *Staphylococcus species, Escherichia coli, Salmonella species, Enterococcus species, Acinetobacter baumannii,* and *Clostridium difficile* (26,27). Among these organisms is a group of bacteria termed ESKAPE, which are responsible for outbreaks in humans and veterinary clinical settings globally (28,29). These bacteria include *Enterococcus faecium, S. aureus, K. pneumoniae*, *A. baumannii, P. aeruginosa*, and *Enterobacter* species (ESKAPE) (14,16,28). This ESKAPE group deserves special attention because they are known to escape the biocidal action of antimicrobials and are associated with increased mortality, morbidity, and healthcare costs in both human and animal medicine (14,29,30). In addition, the World Health Organization (WHO) has listed this group as among the pathogens for which urgent antimicrobial therapy is required due to their tendency to exhibit a high prevalence of multidrug resistance (MDR) (28,29). As such, it is crucial that veterinary medicine also increase the understanding of the virulence, resistance, transmission, and pathogenicity of these bacteria.

2.1.3 Bacterial identification and characterization

These bacteria associated with HAIs can be laboratory identified traditionally by culture and biochemical tests (31). Although the traditional method is still widely used, including, morphology, physiology, chemistry, and biochemical characterization, it is timely, labour

intensive, and not useful enough to identify unambiguously the microorganism to its species level or strain level (31).

The recent advancements in technology have introduced high-tech methods that allow molecular-based techniques, such as the 16S rRNA polymerase chain reaction (PCR) sequencing, Real-Time PCR, Random Amplification of Polymorphic DNA-RAPD-PCR, Restriction Fragment Polymorphism-RFLP, Pulsed-Field Gel Electrophoresis- (PFGE), Whole-Genome Sequencing (WGS), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (31,32).

2.1.3.1 Phenotypic methods

Identification of bacteria using phenotypic methods is done based on their cellular morphology, gram staining, or specialized staining, by assessing growth requirements such as oxygen, pH, temperature, observing colony morphology, and conducting biochemical reactions using enterotubes, selective, and/or differential media types (32,33).

2.1.3.2 Genotypic methods

Molecular techniques have become popular in detecting and identifying bacterial organisms. In surveillance studies, these methods provide reliable epidemiological data that can be used to help trace infections including foodborne disease outbreaks. Most molecular methods are based on DNA analysis, either through amplification or sequencing. Most molecular approaches are DNA-based, utilizing either amplification or sequencing (31). These methods vary from relatively simple DNA amplification-based techniques, such as PCR, real-time PCR, and RAPD-PCR, to more complex ones that use restriction fragment analysis, targeted gene and whole-genome sequencing, and mass spectrometry (31). In addition, approaches based on unique protein signatures, such as MALDI-TOF-MS and similar variations, have also been explored (31,33). In this study, PCR was used for the characterization of ESKAPE pathogens.

2.1.3.3 Polymerase Chain Reaction

Polymerase Chain Reaction is an automated laboratory technique that enables researchers to produce millions of copies of a specific DNA sequence in about two hours and analyzed by gel electrophoresis. Short synthetic DNA fragments called primers are important in this technique because they are used to select a segment of the genome to be amplified, and then multiple rounds of DNA synthesis to amplify that segment. Unlike traditional methods that require bacteria to amplify DNA, PCR is quicker, more efficient, and doesn't require a living organism. It is particularly useful in quickly identifying the causative agent(s) of infections, which is crucial in determining effective treatment intervention (33–35).

Polymerase Chain Reaction is a standard method for identifying bacterial DNA through amplification of the 16S rRNA gene, used in both laboratory and clinical settings. The 16S rRNA gene is highly specific to each bacterial species, making it an ideal target for identification. Although it is a reliable and straightforward method in research laboratories, various factors may influence the results of PCR when applied to clinical settings (31).

2.1.3.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing is mainly used to guide clinical therapy and the results are interpreted using clinical breakpoints. It can also be done to establish patterns of susceptibility in selected organisms using epidemiological breakpoints as a cut-off. In clinical cases of organisms associated with HAIs, clinical breakpoints help identify alternative antibiotics, especially in MDR organisms which are significantly associated with high morbidity and mortality (36).

2.1.4 Sources of organisms associated with hospital-acquired infections

Bacteria associated with HAIs have been identified from several sources including colonized patients (37–39), HCWs (40), commonly used equipment (40–42), HCWs' protective wear (43), and contaminated environments (44,45) within the veterinary hospitals. Since some of the infected patients do not show clinical signs, contaminated environments and

asymptomatic carriers remain the most important sources of bacteria associated with HAIs in veterinary settings (45). As animals tend to explore with their noses and mouths, they may come into contact with contaminated environmental surfaces (45,46). Moreover, environmental surfaces have been found to harbour *E. coli*, Methicillin- Resistant *Staphylococcus aureus* (MRSA), Methicillin- Resistant *Staphylococcus pseudintermedius* (MRSP), and *C. difficile* known to cause HAIs and zoonotic diseases (40,45,47–50). Contaminated personnel and fomites have also been linked to the transmission of HAIs in equine clinics (46,51).

2.1.5 Zoonotic aspect of organisms associated with hospital-acquired infections

The close interaction between humans, animals, and the environment increases the risk of transmission of infectious agents in hospital settings (52). Of great concern is that healthcare workers (HCWs) can carry or be infected with some of the organisms associated with HAIs. Furthermore, handling of infected patients, animal bites, and injuries at work may further increase the risk of exposure to HCWs' and animal owners to zoonotic diseases (53). Furthermore, HCWs may also carry organisms associated with HAIs to their households, resulting in infection at the household level and, subsequently, spreading it into the community (26,54–56). Moreover, Singh et al (43) have isolated MRSA and MRSP from the clothing worn by HCWs during patient care in a veterinary Hospital in Canada. Similarly, pet owners can also be infected with organisms associated with HAIs when visiting a veterinary hospital (57).

Summary

Given that humans, the environment, and animals, in veterinary settings, are reservoirs for agents associated with infectious diseases, it is crucial to prioritize a comprehensive intervention package, that includes but is not limited to prudent anti-ineffective use of drugs, vaccination, antimicrobial stewardship, infection prevention and control practices and identification and isolation of high-risk patients. (3). Implementing these measures helps reduce the spread of infections, as emphasized by Andeson et al (52), Machado et al (4) and Sebola et.al (58).

2.1.6 Control of organisms associated with hospital acquired infections: Antimicrobials

Antimicrobials are a treatment of choice in HAI cases (25). Depending on the organism involved, the most used antibiotics belong to the following groups: penicillins, aminoglycosides, third and fourth-generation cephalosporins, tetracycline, fluoroquinolones and sulfonamides (59). However, persistent infections from HAI-associated pathogens have resulted in the misuse and overuse of antibiotics (59).

2.1.6.1 Antimicrobial resistance among hospital-acquired infections in veterinary Intensive care unit

The high proportion of antimicrobials used is in the ICU makes it a suitable place for the presence of multidrug-resistant organisms (60). The intensive care unit in humans and veterinary hospitals is regarded as a commonplace for the occurrence of antimicrobial resistance (AMR) pathogens (17,60). This is because most patients have high morbidity therefore requiring antimicrobial therapy (60). The ICU is also an interface for patients and environmental surfaces which is known to harbour MDR pathogens. Among the MDR pathogens that have been reported in veterinary medicine especially the ICU are MRSA and MRSP, Extended Spectrum β- lactamase- *E. coli, Salmonella, Enterococcus* spp., *C. difficile*, *A. baumannii,* and *E. coli* (26,27) **(Table 2.1)**.

Bacteria responsible for HAIs may contain resistance genes and virulence factors that enable them to be resistant to antibiotics and survive on different hospital surfaces for longer periods, leading to limited treatment options and worsening the patient's prognosis (8–10,26). These features can either be inherent or acquired from other-resistant bacteria or as a result of selection pressure. For example, an inherent resistance has been reported in β-lactam resistance associated with the presence of the penicillin-binding protein (PBP2a) encoded by the *mec*A or *mec*C gene (26). Whereas, acquired resistance has been reported in *E. coli* associated with plasmids encoding ESBL genes (61,62).

2.1.6.2 Mechanisms of resistance

Antibiotic resistance is mediated by several mechanisms including modification of the target sites, enzymatic inactivation, active efflux, and decreased influx of drugs (63,64). Although organisms may possess more than one mechanism of resistance, there seem to be resistance mechanisms that are agent-specific, for example, resistance to macrolides occurs due to a modification in the ribosomal target. While a mutation in penicillin-binding proteins leads to resistance to β-lactam antibiotics. Similarly, aminoglycoside resistance is mainly enzymatic inactivation (64). In addition, pathogens may acquire resistant genes from other organisms through plasmid-mediated genetic transfer (25).

Although gram-negative and gram-positive bacteria use similar mechanisms of resistance, they differ based on their cell structure and physiology. For example, gram-negative bacteria compared to gram-positive have an outer membrane barrier that can alter porins making them more resistant to many antibiotics. The ability of the bacterium to possess several mechanisms of resistance as indicated above makes it resistant to multiple antimicrobials therefore multidrug resistant (65).

433 **Table 2. 1:** Antibiotic resistance mechanism of gram-positive and gram-negative bacteria

Bacteria	Mechanism of resistance	Example of antibiotics	Reference
Gram- Positive bacteria			
S. aureus and Enterococcus spp.	Use of efflux pumps to actively expel antibiotics from the cell.	Various antibiotics	(25,64–66)
S. aureus	Produces extra Penicillin-binding proteins like PBP2a, which reduces affinity for penicillin and β-lactams.	B-lactams	(25,64–66)
S. aureus and Enterococcus spp.	**Modification of antibiotic target sites**		(25,64–66)
	Modification of the ribosomal binding sites	Macrolides, clindamycin	
	Mutation of genes encoding DNA gyrase and topoisomerase IV	Fluoroquinolones	
	Enzymatic inactivation		(25,64–66)
	β-lactamases hydrolyses β-lactams	β-lactams	
	Production of aminoglycoside-modifying enzymes such as acetyltransferases, phosphotransferases, and adenyltransferases that modify aminoglycosides	Aminoglycosides (gentamycin, tobramycin, kanamycin)	
	Acquisition of resistance genes		(25,64–66)
	Erm (erythromycin resistance) and *msr* (macrolide efflux pump)	Macrolide and lincosamides	
	Tet genes such as *tet*M *and tet*L	Tetracyclines	
	Mutation in the 23S rRNA genes reduces the binding affinity of of the antibiotic to the ribosome	Linezolid	(25,64–66)
Enterococcus species	Mutation in Penicillin-binding proteins and hypersecretion of β-lactamases reduce affinity for β-lactams.	B-lactams	(25,64–66)
Gram- Negative bacteria			
Enterobacteriaceae species	**Synthesis of β-lactamases**	Third generation cephalosporins	(25,64–66)
A. baumannii *P. aeruginosa*	ESBLs	Broad spectrum cephalosporin Monobactams	(25,64–66)

		Penicillins	
	Class A β-lactamases such as SHV-1, TEM-1, and TEM-2	Ampicillin, Amoxicillin	(25,64–66)
	Production of the β-lactamases	Carbapenems	(25,64–66)
	Overexpression of the multidrug efflux pumps	A variety of antibiotics including Imipenem	(25,64–66)
	Production of aminoglycoside-modifying enzymes such as acetyltransferases, phosphotransferases, and adenyltransferases that modify aminoglycosides	Aminoglycosides	(25,64–66)
	Alteration of porins which are proteins found in the outer membrane	Carbapenems	(25,64–66)

434

435 *2.1.6.3 Antimicrobial genes in bacteria associated with HAIs in veterinary medicine.*

436 Numerous antimicrobial genes are present in gram-negative and gram-positive
437 bacteria associated with HAIs (25). In *C. difficile,* the *Gyr*A subunit confers resistance to
438 fluoroquinolones (59), while the presence of erythromycin ribosomal methylase (*ermB*) genes
439 confer resistance to both erythromycin and clindamycin among *Staphylococcus*, *C. difficile*
440 and *Enterococcus* species (67). The *Tet*M gene has been shown to confer resistance to
441 tetracyclines, while *optrA* phenicols in both *E. faecalis* and *E. faecium* have been reported to
442 confer resistance to oxazolidinones. Methicillin resistance among *Staphylococcus* species is
443 associated with the presence of *mec*A gene (47,49,50,67). Additionally, the presence of *cfr*
444 gene in staphylococci has been linked to resistance to linezolid. While the presence of aac(6')-
445 Ib gene confers resistance to aminoglycosides (27). Xia, Gao and Tang (25) have also shown
446 that the colistin resistance in *E. coli* is associated with the presence of *mcr*-genes.

447 **Summary**

448 The presence of antimicrobial genes among organisms of animal origin remains an
449 important issue not only for patient care but also for public health (25). Moreover, Pirš et al
450 (59) have demonstrated that resistant pathogens (*C. difficile*) can be transmitted from infected
451 animals to susceptible human hosts. This study is not unique as other studies have also
452 reported other MDR organisms including MRSA and *E. coli* known to cause diseases in
453 humans (26,40,45,54,55). Measures must be implemented in veterinary medicines to reduce
454 the likelihood of transmission of resistant genes by implementing infection prevention and
455 control (IPC), prudent use, and effective patient management systems (59,68).

456

2.1.7 Infection prevention and control in the control of organisms associated with hospital-acquired and zoonotic infections

The WHO has put together regulations on the core components of IPC practices required to improve the quality and safety of health service delivery and patient health outcomes (69). These practices remain the cornerstone of patient care and management in human medicine (68) and are effective measures addressing HAIs (26,70–72). They have slowly been adopted in veterinary medicine (72,73). In veterinary medicine, IPC practices are designed to protect patients, animal owners, personnel, and communities from HAIs and zoonotic diseases (21,72,73). In addition, they help reduce the burden of pathogens on the hospital environment and the overuse of antimicrobial agents (68,74). Hand hygiene, environmental control, sharps management, vaccination for zoonotic infections, and personal protective equipment (PPE) have been recommended as some of the IPC measures that can be implemented in small animal veterinary hospitals (73). Furthermore, patient management (9,56,75) and surveillance (9) have been described as effective IPC measures in preventing the transmission of organisms associated with HAIs and zoonotic infections. Therefore, personnel and visitors at the veterinary facilities must familiarise themselves with infection control policies (26,74,76–78).

2.1.7.1.1 Environmental control

Several organisms have been isolated from environmental surfaces including bed sites, intravenous stands, surfaces of lockers, and over-bed tables in veterinary medicine (45,47,49,79) as well as on environmental fomites such as cage doors, computer keyboards and mice, stethoscopes, thermometers, and mouth gags (79,80). Some of these pathogens including *C. difficile* are resistant to commonly used disinfections (21,45,54,79) and able to survive on hospital surfaces for a longer period (up to five months) and remain a source of infection to susceptible patients (45,54,79). Therefore, environmental surfaces should be cleaned regularly with disinfectants that are virucidal, bactericidal, mycocidal, non-irritant, non-corrosive, and non-staining (9). Applications may include prepackaged wipes containing

disinfectants such as accelerated hydrogen peroxide or quaternary ammonium compounds or footbaths at the entrance of high-risk areas such as isolation and colic wards/units (79).

Hospital linens and animal bedding, when soiled, pose a threat of infection to patients and staff. They may also contribute to environmental cross-contamination. As a result, any reusable linens and bedding contaminated with bodily fluids or exudates, such as blood, urine, or faeces, must undergo a decontamination process, as outlined in hospital cleaning protocols. Moreover, individuals responsible for handling soiled PPE must receive adequate training (79).

2.1.7.1.2 Patient management

Patient management is another key part of minimizing the incidence of HAIs in veterinary settings (21,75). This area of IPC focuses on patient admission, housing, diagnostic procedures, and treatment. It seeks to identify high-risk patients to prevent transmission of infectious agents in the hospital (56,75). This can be done by strict movement control of patients between services such as diagnostic imaging and surgery (21,75). Where possible, isolation facilities must be available in the hospital to prevent transmission of pathogens from high-risk patients to low-risk patients or to quarantine high-risk patients while waiting for laboratory results (41,81). Patients showing clinical signs or with known bacterial infections must be identified and isolated (56,82). Moreover, effective management of patients with infectious conditions is likely to protect veterinary staff, volunteers, and animal owners from the risk of infection (81).

2.1.7.1.3 Surveillance

Surveillance is the systematic collection, analysis, and interpretation of health data necessary for public health planning, implementation, and evaluation (9). The WHO and the World Organization for Animal Health (WOAH) have developed guidelines for disease surveillance (71,83). This approach can be used to gather data on HAIs and zoonotic diseases. It can also be used to identify critical areas in the hospital and develop strategies for intervention. For example, studies have shown that implementing disease surveillance in

the ICU is important in reducing the risk of HAIs (9,61,72). The surveillance may also be used to monitor the occurrence of MDR organisms (83).

Microbiological surveillance of the hospital environment surfaces is a critical component of a successful infection control program. Hospital environment surveillance can be done either as culture-based, meaning that a sample is taken and processed for results. Or nonculture-based, meaning that the activity relies on the observation of situations and their various outcomes. Either way, surveillance, when implemented correctly, can be a useful and meaningful aspect of a complete hospital infection control program (79).

The South African National Department of Health together with other government agencies developed the South African Antimicrobial Resistance Strategy Framework from 2014 to 2024. This framework consists of five strategic objectives supported by four key enablers, which include optimizing surveillance and early detection of AMR and antimicrobial usage, enhancing infection prevention and control and biosecurity, and promoting appropriate use of antimicrobials in human and animal health through antimicrobial stewardship (84). The framework encourages the sharing of data between laboratories to improve understanding of trends and resistance patterns in the country across animal, human, and environmental health. Furthermore, the framework emphasises a need to build expertise that will incorporate the interventions to tackle AMR in the curricula of undergraduate and postgraduate healthcare professionals. This could be done in collaboration with the health professional councils and training institutions and aligned with relevant WOAH recommendations such as the Veterinary Core Curriculum (85). In 2021, data on the surveillance of AMR and consumption of antimicrobials from human and animal medicine demonstrated sufficient information from human medicine and food-producing animals, but limited data from companion animals (86). With research being among the enablers in the strategic framework, priority research areas including antimicrobial stewardship, IPC and healthcare behaviour change must be prioritised.

2.1.7.1.4 Antimicrobial stewardship

The rapid increase in antimicrobial resistance among organisms associated with HAIs in small animals is concerning (9,56). To combat this development, hospitals should implement clear antimicrobial prudent use policies including bacterial culture (9,75). These policies should be tailored for each veterinary practice or hospital. Veterinarians should ensure correct prescribing following the manufacturers' instructions and where applicable adjust antimicrobial therapy following the results of the antibiogram (9,84). In addition to mitigating the risk of antimicrobial resistance, the prudent use of antimicrobials reduces the risk of adverse events including disruption to commensal flora, promotion of resistance, colonization or infection with opportunistic pathogens, patient toxicity, and drug reactions including anaphylaxis (56). The risk assessment must be conducted to ensure the responsible use of antimicrobials and make recommendations on the current mitigation strategies (84).

2.1.7.1.5 Personal protective clothing

Hospital protective clothing is designed to protect workers from occupational health hazards including bacteria. However, if incorrectly managed it may become a source of pathogens and increase spread in veterinary hospitals (43). Most zoonotic diseases, including ringworm and diarrhoea, are transmitted via direct contact with contaminated body surfaces, body fluids, or a fecal-oral route involving contaminated hands or clothing (43,73,82). For example, MRSA has been reported on personnel clothing working in a veterinary teaching hospital (43). Furthermore, PPE can potentially act as a source of bacteria associated with community-acquired infections (43). HCWs must adhere to appropriate PPE when handling animals with potentially infectious conditions as well as when handling apparently healthy animals (82). Therefore, veterinary hospital infection control protocols must include changing of protective clothing whenever soiled, or when leaving the hospital.

2.1.7.1.6 Sharps management

Sharps mishandling has been associated with high incidences of needle injuries that can subsequently lead to transmission of zoonotic disease (73,82). Wright et al (82) have

reported a high incidence of needlesticks among HCWs who do not recap needles compared to those who recap their needles before disposal.

2.1.7.1.7 Vaccination

Vaccination is an important part of IPC in companion animals. It is important in reducing the risk of infections among both patients and HCWs, especially against zoonotic diseases such as Q- fever and Rabies (56,73,73,82). In such cases, appropriate measures for infection control should be taken, including transmission precautions to prevent accidental exposure to saliva (56).

2.1.7.1.8 Hand hygiene

Transmission of most organisms associated with HAIs including MRSA is mostly through contaminated hands of HCWs (54,55). Studies in both human and veterinary medicine have demonstrated that hand hygiene practices such as hand washing using water and soap and disinfecting hands reduce the transmission of HAIs and antimicrobial-resistant pathogens (54,77,78,80). It remains the cornerstone of IPC in the intensive care unit (54,55,77). Despite this, hand hygiene compliance rates in veterinary hospitals are often low (55,76,82). Willemsen et al (73) reported low hand hygiene compliance among veterinarians, with high compliance after dirty procedures while low compliance was mainly before clean procedures such as needle administration or before patient care. Similarly, in a video observation, overall hand hygiene compliance was found to be 14% in companion animal hospitals in Ontario (87). Veterinary healthcare workers have attributed the low compliance to high workload (55,81), lack of reachable hand washing resources, underlying clinical conditions like skin irritation, forgetfulness, and inadequate knowledge about hand hygiene practices (55,73,76,78,80). To improve hand hygiene compliance, most studies suggest the use of alcohol-based sanitizers as it is less irritating on the skin and requires less time compared to washing hands with water and soap (54,88). Notwithstanding the effectiveness of alcohol-based sanitizers, mechanical hand washing with running water and liquid soap should be used where possible as alcohol-

based sanitizers are less effective against certain pathogens including spore-forming bacteria (54,78).

2.1.7.2 Methods to evaluate Infection Prevention and Control.

The ability to monitor and evaluate infection control practices is important from both research and infectious disease management perspectives. Regular audits in healthcare facilities are to be conducted to help maintain good IPC practices. It involves checking current practices against published national standards of practice (89). Audits of veterinary facilities should include observation of daily practices by hospital staff and assessment of their knowledge of infection control principles and policies, evaluation of the physical facilities, and review of the hospital's written infection control and patient management protocols (80,89,90). Direct observations can be done by an observer on the clinic floor. However, direct observation is prone to bias due to knowledge and experience of the assessor, so it may suffer from "Hawthorne effect" (91,92). Video observation can also be done, however, its feasibility has not been evaluated in small animal veterinary clinics (87,93). The observations made in the audits are then compared with national standards for compliance with standards and identifying areas for improvement. The final step in any audit is planning and implementing changes that will improve practice and commending areas in which infection control principles and practices are implemented well. Communicating audit findings to clinic staff in group meetings is an effective means of keeping everyone invested and working together to maintain good infection control practices (75,79).

Hand hygiene compliance in healthcare facilities can be monitored through direct or indirect methods (94). Direct monitoring involves directly observing hand hygiene practices during patient care. Indirect monitoring involves tracking the usage of hand hygiene products such as liquid soap, hand rub sanitizers, and automated hand rub dispensers (94). Direct observation is considered the gold standard for assessing hand hygiene compliance (71,92,95). It includes an evaluation of HCWs' compliance with the five hand hygiene moments. The five hand hygiene moments as described by the WHO are, (1) before patient

contact, (2) before an aseptic procedure, (3) after contact with body fluids, (4) after touching a patient, and (5) after contact with patient surroundings **(Table 2.2)**. In addition, the antimicrobial-resistant profile of organisms present in the hands of HCWs can assessed before and after an intervention (15,96,97). Microbial sampling can also be done to collect baseline data at any of the five moments of hand hygiene for example before washing hands after washing hands (15), and before contact with patients in both pre- and post-intervention (97). This allows for evaluation of the effectiveness of intervention strategies. The glove juice method is predominantly used in most human studies to quantify the bacterial load on the hands of HCWs as it is more sensitive than the imprint methods (98). For example, is the evaluation of the impact of an intervention to minimise contamination of Vancomycin-Resistant Enterococci (VRE) (99).

2.1.7.3 Challenges of implementing infection, prevention, and control in veterinary hospital

Several factors are known to impact the implementation of IPC measures in veterinary settings. These factors are classified into systematic, organisational, environmental, and individual (100). Systematic factors include material and human resource issues, and policies that affect the implementation of IPC measures. Studies have also reported a lack of written protocols as a barrier to implementing IPC practices in veterinary clinics and hospitals (56,101). The lack of IPC protocols may reduce the standard of care, which can result in legal, ethical, animal health, and occupational health challenges (56). Organizational factors relate to managerial style and support, interprofessional relationships, and budget. Infection prevention and control leaders are considered important in maintaining progress in reducing the risks of HAIs and achieving continuous quality improvement in the hospital (102). Environmental factors relate to the physical layout of the hospital, availability of isolation rooms and hand hygiene equipment such as hand washing basins and access to hand sanitizers, as well as access to PPE (101,103). The individual or personal factors relate to the knowledge, attitudes, and beliefs of healthcare workers about IPC. Studies have associated the behaviour of HCWs during patient care as a contributor to the insufficient implementation of IPC (55,100).

These have been attributed to high workload, lack of resources, underlying clinical conditions including skin irritation, forgetfulness, and lack of knowledge on hand hygiene practices (76,78,80,104). For example, Nakamura and colleagues (55) in the USA, reported that less than 50% of veterinary technicians and veterinary support staff regularly wash their hands every time between handling patients and most of them said that this is because of their busy. Schedules. In addition, Willemsen et al (73) mentioned in a review that financial costs, lack of perceived risk, lack of time for HCWs, and finding a medical practitioner are some of the barriers to implementing an effective IPC in small animal veterinary practice.

2.1.8 One health approach

The One Health approach is a collaborative, multisectoral, and transdisciplinary approach that recognizes the interconnectedness of human health, animal health, and environmental health. It emphasizes the need for cooperation among various disciplines, including medicine, veterinary science, ecology, public health, environmental science, and others, to address complex health challenges effectively (6). One health approach addresses issues such as:

1. **Interconnectedness of Human, Animal, and Environmental Health**: The interconnectedness of humans, animals, and the environment can allow for the emergence of diseases, emphasizing the importance of understanding and addressing these connections (105–109)**.**

2. **Prevention and Control of Zoonotic Diseases**: Many infectious diseases are zoonotic, meaning they can be transmitted between animals and humans. Examples include Ebola, Zika, and COVID-19. The One Health approach emphasizes collaborative efforts to prevent and control such diseases at their source, often involving surveillance, early detection, and coordinated responses across sectors (105–108).

3. **Antimicrobial Resistance (AMR)**: The misuse and overuse of antimicrobial drugs in both human and veterinary medicine contribute to the development of antimicrobial resistance.

One Health recognizes the shared responsibility in addressing this global health threat and advocates for coordinated efforts to promote prudent antimicrobial use, antimicrobial stewardship, and surveillance of antimicrobial resistance in humans, animals, and the environment. (105–108).

4. **Environmental Health**: Environmental factors such as pollution, habitat destruction, climate change, and biodiversity loss can impact human and animal health. One Health emphasizes the importance of understanding and mitigating these environmental stressors to protect the health of all species and ecosystems (105–108).

5. **Food Safety and Security**: The safety and security of the food supply are essential for both human and animal health. One Health approaches integrate efforts to ensure the safety of food production, processing, and distribution systems, considering the health implications for consumers, producers, and the environment (105–108).

As a way to support countries in taking a One Health approach to address zoonotic diseases, the tripartite organizations (FAO, WHO, and WOAH) have jointly developed a guide to use for other health threats and the human-animal-environmental interface such as AMR referred to as Tripartite Zoonotic Guide (TZG)(110).

2.1.9 Multimodal approach

Hand hygiene compliance remains a challenge and long-lasting improvements are difficult to sustain (58,70,93,94). The multimodal approach has been used in fields such as developing technology (111), adaptability to change in the education sector (112), and adaptability to treatments in health and they have proven to be effective compared to a single approach (17,113) in healthcare settings (54,77,78,114).

The World Health Organization (WHO) recommends multimodal approaches as an intervention strategy for sustained improvement of hand hygiene compliance (69,71). The goal is to make hand hygiene a part of the healthcare facility's culture (71) **(Table 2.3)**. There are five key components of the multimodal strategy, which are, system change, training/education,

evaluation and feedback, reminders in the workplace, and institutional safety climate (71). In addition, the WHO outlines the five step-by-step approaches to implement the multimodal approach to improve hand hygiene (71). The steps include facility preparedness (readiness for action), baseline evaluation (establishing knowledge of the current knowledge), implementation (introducing the improvement activities), follow-up evaluation (evaluating the implementation impact), and ongoing planning and review cycle (developing a plan for the next five years minimum) (71). Smith et al (104) have included four of the five approaches by the WHO to the ongoing planning and review cycle to improve hand hygiene compliance of HCWs in the ICU of a small animal veterinary teaching hospital of Georgia College. The results of the study showed no significant improvement in overall compliance with hand hygiene, which was below 50% pre-and post-intervention.

The five key components together with the five key approaches and their relevant tools encourage the HCWs to comply with the five moments for hand hygiene which are: (1) before patient contact, (2) before an aseptic procedure, (3) after contact with body fluids, (4) after touching a patient, and (5) after contact with patient surroundings **(Table 2.2)** within health facilities. Studies have shown that healthcare workers are compliant to hand hygiene after contact with bodily fluid which is typically intended to protect HCWs from infections and not to protect patients (104).

2.1.9.1.1 System change

Hand hygiene improvement tools and improvement of existing infrastructure can be used at the start of the journey to improve hand hygiene compliance within hospitals. These tools can also be used for routine or periodic monitoring of product use and infrastructure (71).

*2.1.9.1.2 T*raining and education

Education plays a crucial role in implementing effective hand hygiene strategies and it is strongly linked to the other five key components of IPC multimodal strategies. In fact, without proper training, it is unlikely that any system changes will result in behavioral changes, such as adopting the use of alcohol-based hand rubs and maintaining proper hand hygiene.

Training can differ depending on the need, in some cases, education on basic principles might be required and in some complex cases practical scenarios may be used to apply theoretical principles (71). Amongst all preventative methods, education is often the first IPC intervention strategy to ensure compliance with IPC protocols (78,114). Educating personnel and clients on HAIs and zoonotic risks help reduce the incidences of these diseases (76). Salama et al. (77) in Kuwait reported an improvement in hand hygiene compliance among healthcare workers after an educational campaign from 43% to 61.4% compliance respectively. Therefore, personnel and visitors in the veterinary facilities must be familiar with infection control policies (26,74,77,78).

2.1.9.1.3 Evaluation and feedback

Hand hygiene compliance in healthcare facilities can be monitored through direct or indirect methods (71). Direct monitoring involves directly observing hand hygiene practices during patient care. Indirect monitoring involves tracking the usage of hand hygiene products such as soap, hand rub, and automated hand rub dispensers. Direct observation is considered the gold standard for assessing hand hygiene compliance with an emphasis on the five hand hygiene moments (71).

Knowledge among HCWs must be assessed to gather baseline information in order to determine the need for intervention that may include education and training. The results of the assessment of HCW's knowledge can be disseminated through written reports or communicated during meetings or hospital-structured feedback sessions (71). In South Africa, evaluation of the IPC practices has been done in human medicine with no study evaluating IPC practices in veterinary medicine. One such study is that by Mehtar et al. (115) which evaluated infection control practices in public dental care services. This study reported a lack of knowledge among the HCWs on the application of IPC in clinical practice and suggests this is likely to increase the risk transmit blood-borne viruses in public dental facilities. If a healthcare facility cannot provide sufficient hand hygiene training due to resource constraints such as a lack of trained facilitators and finances especially in resource-limited facilities, an

action plan should be developed to embed training and education within the facility's culture (71). Once the interventions have been implemented a follow-up assessment must be undertaken to assess the effectiveness of the intervention strategies on hand hygiene compliance.

2.1.9.1.4 Reminders in the Workplace

The use of reminders and communication tools is crucial in the workplace to prompt and remind healthcare workers about the significance of hand hygiene, proper indications, and procedures for performing it. These tools also serve as a means of educating patients and visitors about the standard of care that they should anticipate from healthcare workers (71). Reminders can be done in the form of posters, this has to be done with caution as Anderson et al (87) have reported limited improvement in hand hygiene compliance after the poster campaign initiative.

2.1.9.1.5 Surveillance

Furthermore, several studies in human hospitals indicated a need for a better understanding of the number of antimicrobial-resistant organisms present on hands and to identify ways to improve adherence to hand hygiene practices. During these campaigns, HCWs' hands were sampled for culture before and after multimodal campaign interventions during different stages of patient care to measure the difference in microbial load (15,96,97). The five moments of hand hygiene were also noted in this campaign, for example, Matuka et al (15) isolated *Staphylococcus* spp. a*nd E.* coli from sampling HCWs before washing hands and after washing hands. Monistrol et al (97) observed a reduction in both resident and transient flora counts on the hands of HCWs sampled before contact with patients during an educational intervention. While Tenorio et al (99) demonstrated the effectiveness of glove use in minimizing contamination of Vancomycin-Resistant Enterococci (VRE) during patient care.

2.1.9.2 Multimodal approach case studies in veterinary hospitals

Although common in human healthcare facilities, some veterinary facilities have also implemented multimodal approaches to improve hand hygiene with differing success. Shea and Shaw (76) in a small animal hospital in the United States of America, observed a significant increase in hand hygiene compliance after an education campaign, from 20.6% to 41.7%. Contrary to the study by Shea and Shaw, Smith and colleagues (81) at a small animal hospital in the United States of America observed no significant improvement in hand hygiene compliance after an intervention. Instead, glove donning was a confounding matter in the study. Similarly, Anderson et al (87) have limited improvement in hand hygiene compliance after the poster campaign initiative.

Table 2. 2: This table illustrates the World Health Organization multimodal hand hygiene improvement strategy and tools for implementation. Source (71).

Tools for System Change	Tools for Training / Education	Tools for Evaluation and Feedback	Tools for Reminders in the Workplace	Tools for Institutional Safety Climate
Ward Infrastructure Survey	Slides for the Hand Hygiene Co-ordinator Slides for Education Sessions for Trainers, Observers and Health-Care Workers	Hand Hygiene Technical Reference Manual	Your 5 Moments for Hand Hygiene Poster How to Handrub Poster	Template Letter to Advocate Hand Hygiene to Managers
Alcohol-based Handrub Planning and Costing Tool Guide to Local Production:	Hand Hygiene Training Films	Observation Tools: Observation Form and Compliance Calculation Form	How to Handwash Poster	Template Letter to Communicate Hand Hygiene Initiatives to Managers
WHO-recommended Handrub Formulations	Slides Accompanying the Training Films	Ward Infrastructure Survey	Hand Hygiene:	Guidance on Engaging Patients and Patient
Soap / Handrub Consumption Survey	Hand Hygiene Technical Reference Manual	Soap / Handrub Consumption Survey	When and How Leaflet SAVE LIVES:	Organizations in Hand Hygiene Initiatives
Protocol for Evaluation of Tolerability and Acceptability of	Observation Form	Perception Survey for Health-Care Workers	Clean Your Hands Screensaver	Sustaining Improvement – Additional Activities for Consideration by Health-Care Facilities
Alcohol-based Handrub in Use or Planned to be Introduced: Method 1	Slides for the Hand Hygiene Co-ordinator Slides for Education Sessions for Trainers, Observers and Health-Care Workers	Perception Survey for Senior Managers		SAVE LIVES: Clean Your Hands Promotional DVD
	Hand Hygiene Training Films	Hand Hygiene Knowledge Questionnaire for Health-Care Workers		
	Slides Accompanying the Training Films	Protocol for Evaluation of Tolerability and Acceptability of		
	Hand Hygiene Technical Reference Manual	Alcohol-based Handrub in Use or Planned to be Introduced: Method 1		
	Observation Form	Protocol for Evaluation and Comparison of Tolerability and Acceptability of Different Alcohol-based Handrubs: Method 2		
	Health-Care Workers	Data Entry Analysis Tool		
	Hand Hygiene Training Films	Instructions for Data Entry and Analysis		
	Slides Accompanying the Training Films	Data Summary Report Framework		

788 **Table 2. 3:** Definitions of the hand hygiene five moments: Source (94).

MOMENT	WHEN AND WHY
BEFORE PATIENT CONTACT	Healthcare workers in direct contact must perform hand hygiene before touching a patient to remove any potential pathogens that were picked up from previous patients. Even if the healthcare worker does not touch the patient directly, they may encounter a patient's clothing or personal objects with harmful microorganisms
BEFORE AN ASEPTIC PROCEDURE	This moment occurs before any clean or aseptic procedure within a patient zone. A clean procedure may include opening a venous access line, giving an injection, or performing wound care. Importantly, hand hygiene required at this moment aims at preventing hospital-acquired infections. Some procedures on clean sites require glove use. In this case, hand hygiene is required before putting on gloves because gloves alone may not entirely prevent contamination and after removal of the gloves.
AFTER CONTACT BODY FLUID EXPOSURE	Hand hygiene is required instantly after a procedure associated with a risk to expose hands to body fluids. It must take place before any next hand-to-surface exposure, even within the same patient zone. This hand hygiene action may reduce the risk of colonization or infection of healthcare workers with infectious agents that may occur even without visible soiling. Additionally, it may reduce the risk of transmission of microorganisms from a "colonized" to a "clean" body site within the same patient.
AFTER PATIENT CONTACT	Hand hygiene should happen when leaving the patient zone after a care sequence, before touching an object in the area outside the patient zone and before a subsequent hand exposure to any surface in the health-care area. hand hygiene minimizes the risk of dissemination to the health-care environment, substantially reduces contamination of HCWs' hands with the flora from one patient to the other patient and protects the HCWs themselves.
AFTER CONTACT WITH PATIENT SURROUNDING	This moment occurs after hand exposure to any surface in the patient zone, and before a subsequent hand exposure to any surface in the health-care area, even if a patient is not touched.

789

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Chapter 3 Systematic Literature Review

Hospital-acquired and zoonotic bacterial organisms and their associated antimicrobial-susceptibility profile in veterinary hospitals: A Systematic Review

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My contribution to the paper includes study design, data analysis, interpretation of results, writing of manuscript as well as extensive editing of the manuscript.

Abstract

Background: Hospital-acquired infections (HAIs) are associated with increased mortality, morbidity, and an economic burden due to costs associated with extended hospital stays. Furthermore, most pathogens associated with HAIs in veterinary medicine are zoonotic. This study used published data to identify organisms associated with HAIs and zoonosis in veterinary medicine. Furthermore, the study also investigated the antimicrobial-susceptibility profile of these bacterial organisms.

Methods: A systematic literature review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses" (PRISMA) guidelines. Search terms and five electronic databases were used to identify studies published over 20 years (2000-2020). The risk of bias was assessed using the "Strengthening the Reporting of Observational Studies in Epidemiology-Vet" (STROBE-Vet) checklist.

Results: Out of the identified 628 papers, 27 met the inclusion criteria for this study. Most studies (63%, 17/27) included were either from small animal or companion animals' clinics/hospitals, while 5% (4/27) were from large animal clinics inclusive of bovine and equine medicine. Hospital-acquired bacteria were reported from environmental surfaces (33.3%), animal clinical cases (29.6%), and items such as cell phones, clippers, stethoscopes, and computers (14.8%). *Staphylococcus* species. was the most (63%; 17/27) reported organism, followed by *Escherichia coli* (19; 5/27), *Enterococcus* spp. (15%, 4/27), *Salmonella* spp. (15%; 4/27), *Acinetobacter baumannii* (15%, 4/27), *Clostridium difficile* (4%, 1/27), and *Pseudomonas aeruginosa* (4%; 1/27). Multidrug-resistant (MDR) organisms were reported in 71% (12/17) of studies linked to Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), *Enterococcus* spp., *Salmonella Typhimurium, A. baumannii*, and *E. coli*. The *mec*A gene was identified in both MRSA and MRSP, bla_{CMY-2} gene in *E. coli* and *Salmonella* spp., *flo* genes in *E.coli,* and *van*A gene in *E. faecium* isolate. Six studies reported organisms from animals with similar clonal lineage to those reported in human isolates.

Conclusion: Organisms associated with hospital-acquired infections and zoonosis have been reported from clinical cases, environmental surfaces, and items used during patient treatment and care. *Staphylococcus* species is the most reported organism in cases of HAIs and some isolates

shared similar clonal lineage to those reported in humans. Some of the bacteria associated with HAIs exhibited a high level of resistance and contain genes associated with antibiotic resistance.

3.1 Introduction

Hospital-acquired infections (HAIs) in both veterinary and human medicine are associated with increased mortality, morbidity, and are an economic burden due to the increased cost of extended hospital stay and treatment options (1,2). The most reported HAIs include surgical wounds, urinary tract, and gastrointestinal infections (1–3) and are often associated with bacteria such as *Enterococcus* species (spp.), *Escherichia coli*, *Staphylococcus* spp., *Enterobacter* spp., *Klebsiella* spp., *Acinetobacter* spp., and *Pseudomonas* spp.(3–6).

Available evidence suggests that HAIs associated with *Enterococcus* spp., *Escherichia coli*, *K. pneumoniae,* and *S. aureus* are on the increase in veterinary medicine (7,8). There are also reports of vancomycin-resistant enterococci (VRE), multidrug-resistant (MDR) *E. coli*, carbapenem-resistant *Acinetobacter baumannii,* carbapenem-resistant *P. aeruginosa,* carbapenem-resistant and extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae,*(3–6,9–11) with limited treatment options and poor prognosis (1,5,6,9,11). It is estimated that 60% of emerging infectious diseases are likely to come from animals (12,13). Of concern is that bacteria associated with HAIs in veterinary settings could be contributing to the emergence of these new diseases (6,14). Since the veterinary hospital environment is a human-animal interface, it remains a potential source of zoonotic pathogens (6,15). Therefore, veterinary healthcare workers (HCWs) and animal owners are at an increased risk of contracting various zoonotic infections (12,13). This is likely to put financial stress on the human health system especially in developing countries (16). In view of this, continuous surveillance of hospital-acquired and zoonotic pathogens in veterinary medicine should be done to better quantify the risk of transmission to personnel and animal owners (17,18).

Systematic review studies have suggested that improving surveillance systems is critical in the prevention of HAIs and in reducing the emergence of antimicrobial-resistant pathogens (6,19). Therefore, a holistic approach is needed to investigate the types of disease agents, hosts, the antimicrobial-resistance profile of the organism, and the virulence of the organisms associated with HAIs in veterinary medicine (15).

This study describes the occurrence and antimicrobial-susceptibility profiles of bacterial organisms associated with HAIs and zoonosis in veterinary medicine. It addresses the following

research questions: (1) Which bacteria associated with HAIs and zoonotic diseases have been reported in veterinary hospitals? (2) What is the antimicrobial resistance profile of these bacteria?

3.2 Materials and methods

The systematic literature review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (20). Keywords and synonyms used in various databases included hospital-acquired organism or infection, nosocomial organism or infection, animal to animal infections, zoonotic infection, zoonosis, animal to human infections, veterinary hospital, and veterinary clinic.

3.2.1 Information source

Search terms and electronic databases used in this study are provided in **Table 3.1.** Since each database has a different search function, alternate search terms appropriate for each database were used. Boolean operators were utilized in all searches. A data search was conducted between June 2020 and December 2020. A follow-up search was performed in January 2021, however, there were no additional studies considered based on the inclusion criteria. Mendeley reference manager was used to store all studies and documents retrieved

Table 3. 1: Search terms and databases utilized to search for articles included in this review about hospital-acquired and/or zoonotic infections in veterinary facilities between 2000 and 2020.

Publications	Search terms
Science Direct	Veterinary AND "Infection Control" AND "hospital acquired infection OR nosocomial" AND zoonoses OR zoonotic OR zoonosis "Veterinary hospital OR clinic" AND “hospital acquired infections” OR nosocomial AND zoonoses OR zoonotic OR zoonosis "Systematic literature review" AND "Hospital acquired infection OR nosocomial" AND "zoonoses OR zoonosis OR zoonotic" AND veterinary "Hospital acquired infection OR nosocomial" AND "zoonoses OR zoonosis OR zoonotic" AND veterinary Veterinary AND "hospital acquired infection OR nosocomial" "Veterinary hospital" AND "hospital acquired infection OR nosocomial" NOT "Human hospital"
PubMed	"Hospital acquired infections OR nosocomial" AND veterinary AND “zoonosis or zoonoses or zoonotic” "Infection prevention and control" [All Fields] AND veterinary AND “hospital acquired infection or nosocomial” AND zoonoses "Hospital acquired infections OR nosocomial" AND veterinary
Web of Science	"Hospital acquired infections" AND veterinary "Hospital acquired infections" AND "veterinary hospital" "Hospital acquired infections" AND "zoonotic infections" AND "Veterinary hospital"
Google Scholar	"Systematic literature review" AND "Hospital acquired infection OR nosocomial" AND "zoonoses OR zoonosis OR zoonotic" AND veterinary "Hospital acquired infection OR nosocomial" AND "zoonoses OR zoonosis OR zoonotic" AND veterinary "Hospital acquired infection OR nosocomial" AND "veterinary hospital"
Scopus	"Hospital acquired infection" AND zoonoses AND veterinary nosocomial AND zoonoses AND veterinary

3.2.2 Eligibility criteria

Only manuscripts published in peer-reviewed journals were considered for inclusion in this study. Primary research articles written in English and published between 2000 and 2020 were selected. The microbiological data included bacterial isolates from HAIs cases, hospital environmental screening, fomites from veterinary hospitals, and zoonotic cases in veterinary hospitals. In addition, the antimicrobial resistance profiles of the different bacteria were also

extracted. The inclusion and exclusion criteria are listed in **Table 3.2**. Two investigators (DC, DN) independently screened the titles and abstracts from the searches. Any disagreements were settled by discussion.

Table 3. 2: Inclusion and exclusion criteria of articles reporting on hospital-acquired and/or zoonotic infections in veterinary facilities between 2000 and 2020.

Inclusion Criteria	Exclusion Criteria
Veterinary medicine studies	Human hospital studies
Small animal/ Companion animal	Farms, home studies
Equine/ Large animals	
Peer-reviewed research	Reviews
Year 2000- 2020	Policies, Government documents and conference reports, Book chapters
Studies in English	Non-English studies
Infection prevention and control practices (Environmental screening)	

3.2.3 Study selection

3.2.3.1 Data collection process and data items

For each study that met the selection criteria for inclusion, the following data were extracted: author, year, the theme of study (HAIs or zoonotic studies), and the antimicrobial resistance profile.

3.3 Results

3.3.1 Study selection

A total of 628 studies were identified; 330 articles remained after the initial screening. Based on the eligibility screening criteria, 48 studies remained and were further critically assessed. A total of 27 studies met the inclusion criteria and were further analysed **(Figure 3.1)**.

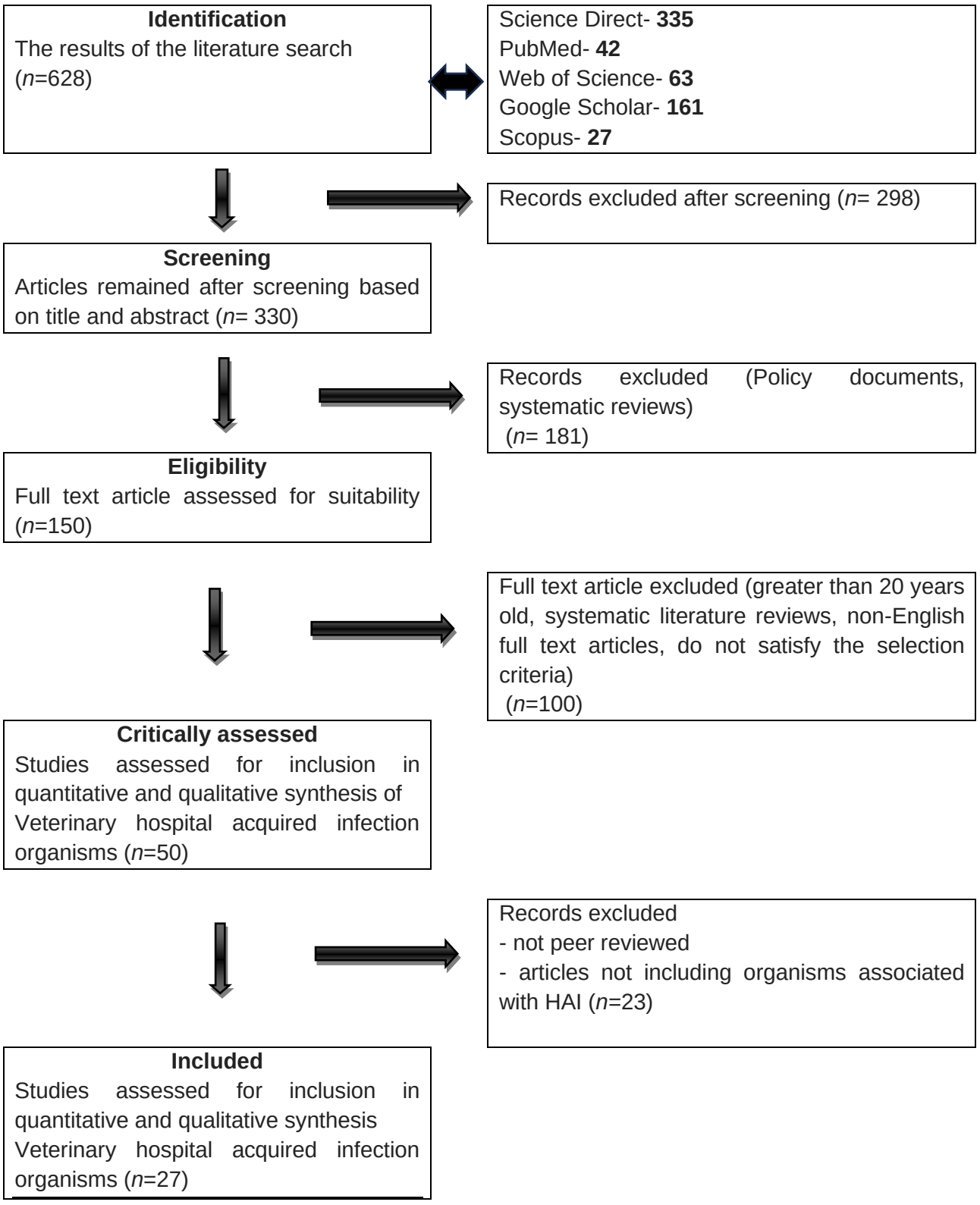

Figure 3. 1: Summary of study selection and exclusion using the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.

3.3.2 Risk of bias

Strengthening the Reporting of Observational Studies in Epidemiology (STROBE-Vet) statement is a 22-item tool that allows a systematic way of reporting on veterinary observational studies. The STROBE statement was developed to guide the reporting of observational studies related to human health. These methods have been adopted and used for standardised reporting guidelines for observational studies in veterinary medicine (19,21). Identified studies that met the inclusion criteria were cross-sectional and cohort studies (21). Each study was assessed individually according to each of the 22 items.

Items were considered to have been reported sufficiently if the studies provided a detailed abstract and clear title (item one), background, and rationale (item two), stated the objectives (item three), presented key elements of the study design (item four), described the sample size (item 11), reported outcomes for the study (items 14&15), provided estimates and parameters (item 16), summarized key results regarding study objectives (item 18 &19), interpreted results (item 20), discussed the results (item 21), and stated the funding source as well as the role of authors as described by Sergeant et al (21).

Only two studies (7%, 2/27) reported on all STROBE-Vet items (22,23). Based on STROBE-Vet, item 1 was partially attained by 19/27 (70%) studies as they excluded the study design and was fully attained by 8 (29%) studies. Items 6, 13,14, and 20 were fully attained by all the studies, Items 2, 4, 5, and 16 were fully attained by 26 (96%) of the studies, items 3,15,17, and were fully attained by 25 (93%) of the studies, item 7 and 18 were fully attained by 24 (89%) of the studies, items 9 and 19 were fully attained by 21 (78%) of the studies, items 11 and 21 were fully attained by 20 (74%) studies and item 10 was fully attained by 63% of the studies. Twelve (12; 44%) studies provided the funding sources, twelve (12; 44%) studies declared no conflict of interest, three studies (3; 11%) mentioned the contribution of each author, and three (3, 11%) provided ethical clearance declarations **(Annexures).**

3.3.3 Sources of data

All the studies reviewed were observational studies. More than half (18; 67%) of the reported studies were cross-sectional studies, three (11%) were case-controlled studies (reported following an outbreak), and six (22%) reported on retrospective data.

Twenty-four (89%) studies focused on a specific bacterium, whereas the other three studies (11%) (15,24,25) reported generally on the bacteria associated with HAIs. Most studies (78%) (17,18,25–43) investigated the occurrence of HAIs in a single facility, five (19%) (3,15,22–24) studies investigated multiple facilities in an area, and one (4%) (44) study did not specify the area of study.

Seventeen (17/27, 63%) studies were from either the small animal or companion animal clinics/hospitals (3,15,18,22–28,32,36–40,44). followed by both bovine (33,37,45,46) (4/27, 15%) and equine medicine (17,42,43). Three (3/27, 11%) studies were a combination of small animals, large animals, and poultry (30,31,35). One (1/27, 4 %) study did not identify the type of veterinary clinic or hospital (34).

Within the hospital settings, bacteria associated with HAIs were reported from environmental surfaces (9/27; 33%) (15,18,31,34,35,37,39,40,43), animal cases (8/27; 30%) (3,17,23,26,28,30,38,43), and commonly used equipment such as clothing, cell phones, clippers, stethoscopes, and computers (4/27,15%,) (24,27,31,36). Only three studies (3/27, 11%) isolated bacteria from humans who have regular contact with animals (15,27,33).

The antimicrobial resistance profile of the different organisms was provided in eighteen (17/27, 63%) studies (3,15,18,23,26,28–32,34,35,37,38,40,42,43), while nine (9/27, 33%) studies did not report on the antimicrobial resistance patterns (17,22,24,25,27,33,36,39,44). Thirteen studies (13/27, 48%) further characterized the microorganisms using pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) assays (3,17,18,23,26,28–30,32,35,41–43).

3.3.4 Bacterial isolates associated with hospital-acquired infections

Staphylococcus spp. Isolates were the most (17/27, 63%) reported pathogens associated with HAIs, followed by *Escherichia coli* (5/27; 19%), *Enterococcus* spp. (4/27; 15%), *Salmonella* spp. (4/27; 15%), *A. baumannii* (4/27; 15%), *C. difficile* (1/27; 4%), and *P. aeruginosa*. (1/27; 4%). *Enterococcus faecalis* (3/4; 75%) and *E. faecium* (3/4; 75%) were the most reported of the *Enterococcus* species.

Among the *Staphylococcus* spp., 11 (11/17, 65%) were MRSA and six (6/17, 35%) were methicillin-resistant *S. pseudintermedius* (MRSP). Three out of five (3/5; 60%) studies reported MDR *E. coli* isolates and one (1/5; 20%) study reported an extended spectrum β-lactamase (ESBL) producing *E. coli*. Meanwhile, vancomycin-resistant *enterococci* were reported in one (1/4; 25%) study. *Salmonella* Typhimurium was reported as the common serotype in two of the four (2/4; 50%) studies. Two of the four (2/4; 50%) studies reported the presence of MDR *Salmonella* **(Table 3.3).**

Table 3. 3: Organism reported in hospital-acquired and/or zoonotic infections in veterinary facilities between 2000 and 2020.

Bacteria	Citation
***Staphylococcus* species**	(3,15,17,18,22,24,25,27–29,31,32,35–37,39,40)
Methicillin-resistant *S. aureus*	(15,18,22,27,30–33,35,36,61)
Methicillin-resistant *S. pseudintermedius*	(15,22,27,36,39,40)
Clostridium difficile	(15)
***Enterococcus* species**	(3,15,37,38)
E. faecalis	(3,37,38)
E. faecium	(3,37,38)
Vancomycin-resistant enterococci	(15)
Acinetobacter baumannii	(3,23,24,34)
Escherichia coli	(15,24,26,34,46)
Extended spectrum β-lactamase (ESBL)	(15)
Multidrug resistance *E. coli*	(15,26,34)
***Salmonella* species**	(15,33,42,43)
Multidrug-resistant *Salmonella*	(42,43)
Pseudomonas aeruginosa	(37)

3.3.5 Sources of organisms associated with hospital-acquired infections

The following pathogens were detected in the hospital environmental surfaces, namely MRSA (15,18,32,35), MRSP (37,39), ESBL-producing *E. coli* isolates (15), VRE (15), *A. baumannii* (34), *C. difficile* (15) and *P. aeruginosa* (37). Common pathogens identified from hospital equipment included: MRSA (17,27,31,36), MRSP (15,36,40), *Enterococcus faecalis* (37), and *A. baumannii* (24,27,34).

Among patients in hospital settings, MRSA was isolated from companion (30) and equine animals (17,29). Multidrug resistant *Escherichia coli* was isolated from companion and bovine animals (26,46). Additionally, *Enterococcus faecium, Enterococcus faecalis* (3,42). and *A. baumannii* (3) were isolated from companion animals. *Salmonella* species were also isolated from patients (33,43), healthy animals (42), and the hospital environment (15,42) **(Table 3.4).**

The healthcare workers (HCWs) harboured MRSA (22,32,37), MRSP (27,37), *E. faecium* (37) and two studies reported MRSA among pet owners (22,29). In addition, van Duijkeren et al (30) and Hoet et al (18) reported on the zoonotic potential of MRSA with van Duijkeren et al (30) identifying MRSA clusters in animals with a similar clonal lineage to that reported in humans **(Table 3.6).**

Table 3. 4: Sources of hospital acquired organisms based on the systematic reviewed papers published from 2000 to 2020.

Source	[a]MRSA	[b]MRSP	[c]ESBL *E. coli*	[d]MDR *Escherichia coli*	*Enterococcus faecalis*	*Enterococcus faecium*	*C. Difficile*	*P. aeruginosa*	*A. baumannii*	*Salmonella* spp.
Animal										
Patients	(30)			(26)	(3)	(3)				(43)
	(29)			(46)	(38)	(38)			(3)	(33)
	(17)									
Healthy Environment										(42)
Hospital	(15)	(37)	(15)				(15)	(37)	(34)	(15)
	(18)	(39)								(42)
	(35)									
	(32)									
Equipment	(17)	(15)			(37)				(24)	
	(31)	(40)							(27)	
	(27)	(36)							(34)	
	(36)									
Healthcare workers	(37)	(37)				(37)				
	(49)	(27)								
	(22)									
Pet Owners	(22)									
	(22,29)									

[a]Methicillin-resistant *Staphylococcus aureus*

[b]Methicillin-resistant *Staphylococcus pseudintermedius*

[c]Extended-spectrum beta-lactamase producing- *E. coli*

[d]Multidrug-resistant *E. coli*

3.3.6 Antimicrobial resistance patterns of bacteria associated with hospital acquired infections

3.3.6.1 Phenotypic resistance

Out of the 27 studies reviewed, 17 (63%) conducted an antimicrobial susceptibility test on the isolates. Among these, 12 (71%) studies reported isolates resistant to more than one antimicrobial. Bacteria resistant to multiple drugs identified included MRSA (18,29,35,41), MRSP (37), *A. baumannii* (23,34), *E. coli* (26,44), *Salmonella* Typhimurium (42,43), *E. faecalis* and *E. faecium* (38).

Methicillin-resistant *Staphylococcus aureus* isolates showed resistance towards ampicillin, amoxicillin, oxacillin, clindamycin, gentamycin, ciprofloxacin, cephalexin, enrofloxacin, cefuroxime, chloramphenicol, erythromycin, and kanamycin while MRSP isolates showed resistance towards azithromycin, oxacillin, penicillin, clindamycin, gentamycin, tetracycline, and ciprofloxacin. *Clostridium difficile* showed resistance towards rifampin, moxifloxacin, and chloramphenicol. *Enterococcus faecalis* and *E. faecium* showed resistance towards ampicillin, tetracycline, ciprofloxacin, enrofloxacin, erythromycin, and rifampicin (38). *Enterococcus faecium* was also reported to be resistant to amoxicillin and vancomycin (37). *Acinetobacter baumannii* exhibited resistance to amoxicillin, tetracycline (34), ciprofloxacin (23) and imipenem (23). While *E. coli* showed resistance to ampicillin, cefoxitin, oxacillin, and penicillin (26,44) and *Salmonella* was resistant to ampicillin, amoxicillin, cefoxitin, gentamycin, tetracycline, chloramphenicol, rifampicin, and streptomycin (43) **(Table 3.5).**

Table 3. 5: Phenotypic antimicrobial resistance profile of hospital-acquired infection organisms based on the systematically reviewed papers published from 2000 to 2020.

PATHOGENS	AMP	AMX	CEF	AZI	OXA	PEN	CLI	GEN	TET	CIP	VAN	CFL	ENF	CFR	CHL	ERY	KAN	RIF	MOX	CLO	CPH	IMI	STR	AMX-C
Gram-positive bacteria																								
[1]MRSA	(30)	(32)	(33)	(37)	(37)	(37)	(32)	(37)		(18)		(30)	(18)	(32)	(32)	(32)	(32)							
	(18)	(30)			(18)	(35)	(18)	(30)		(32)			(30)			(30)								
	(35)	(18)			(35)		(37)	(18)								(18)								
	(38)	(35)														(46)								
[2]MRSP				(37)	(37)	(37)	(37)	(37)	(37)	(37)														
E. faecium	(38)	(37)						(35)	(37)	(37)	(37)		(38)					(38)						
	(37)								(38)	(38)														
E. faecalis	(38)							(35)	(38)	(38)			(38)			(38)		(38)						
C. difficile																		(17)	(17)	(17)				
Gram-negative bacteria																								
E. coli	(44)		(44)		(44)	(44)																		(46)
	(26)		(26)																					(28)
A. baumannii		(34)							(34)	(23)		(23)	(23)									(17)		(23)
Salmonella spp.	(42)	(42)	(42)					(42)	(42)						(42)			(42)			(42)		(42)	(42)
	(43)							(43)	(43)						(43)								(43)	(43)

AMP=Ampicillin, AMX=Amoxicillin, CEF=Cefoxitin, AMX-C=Amoxycillin-Clavulanic Acid, AZI= Azithromycin, OXA=Oxacillin, PEN=Penicillin, CLI=Clindamycin, GEN=Gentamicin, TET=Tetracycline, CIP=Ciprofloxacin, VAN=Vancomycin, LIN=Linezolid, CFL=Cephalexin, ENF=Enrofloxacin, CFR=Cefuroxime, CHL=Chloramphenicol, ERY=Erythromycin, KAN=Kanamycin, CHL=Chloramphenicol, STR=Streptomycin, RIF=Rifampin, IMI=Imipenem, MOX=Moxifloxacin, CLO=Clarithromycin, IMI= Imipenem STR= Streptomycin

[1]MRSA=Methicillin-resistant *Staphylococcus aureus*

[2]MRSP=Methicillin-resistant *Staphylococcus pseudintermedius*

3.3.6.2 Antimicrobial genes

Among *Staphylococcus* species, *mec*A was reported in five MRSA studies(18,30,31,35,45) (33,118–121) and two MRSP studies (37,40). β-lactamase gene (bla_{CMY-2} gene) was reported in *Salmonella* spp. (43) and *E. coli* isolates (15,26,44). While the vancomycin-resistant gene (*vanA* gene) was reported by one *E. faecium* study (37). The *flo* gene was identified in one *E. coli* study (26) **(Table 3.6).**

Table 3. 6: The antimicrobial resistant genes isolated from bacteria associated with hospital-acquired infection bacteria, published data between 2000 and 2020.

Pathogens	*mec*A	bla_{CMY-2}	*flo*	*van*A
[1]MRSA	(27) (31) (35) (30) (45)			
[2]MRSP	(37)(40)			
E. coli		(15) (44) (26)	(26)	
E. faecium				(37)
Salmonella spp.		(43)		

[a]Methicillin-resistant *Staphylococcus aureus*
[b]Methicillin-resistant *Staphylococcus pseudintermedius*

3.3.7 Zoonosis

Six (22%) studies (18,29,30,35,41,46) reported HAI associated organisms that are zoonotic in nature. For example, MRSA with a SCC*mec* type IV isolated in humans(23) has also been isolated in hospitalized horses (41) and hospitalized dogs (35). Similarly, three studies reported clonal MRSA lineage in animals similar to that previously reported in humans (29,30,35). A plasmid DH108/30218 in *E. coli* isolates in animals has also been identified which is similar to a cassette (18-ESBL 188) reported in humans (46)

3.4 Discussion

Hospital-acquired infections and zoonosis are increasingly becoming a global concern (47). In addition, there is an increasing prevalence of resistance among these organisms to commonly used antimicrobials. Most studies that have investigated HAIs and their antimicrobial resistance profiles are in human medicine. In view of this, studies on the occurrence and resistant profile of organisms associated with hospital-acquired and zoonotic infections in veterinary medicine are needed. In this study, bacterial organisms associated with hospital-acquired and zoonotic infections

isolated were identified. Furthermore, most of the organisms identified were multidrug-resistant or harboured resistant genes. Several sources of bacterial organisms associated with HAIs including HCWs, commonly used instruments, equipment, and contaminated hospital environments were also identified.

Bacteria associated with HAIs identified MRSA, MRSP, *Enterococcus* spp., *A. baumannii, P. aeruginosa*, *C. difficile, E. coli,* and *Salmonella* spp., (3,15,18,24,25). The presence of these bacterial pathogens within veterinary settings is a public health concern and emphasises the need for the implementation of infection prevention and control measures to eliminate these pathogens. The patient flora, healthcare workers, commonly used equipment, and the hospital environment were identified as possible sources of organisms associated with HAIs. Therefore, control measures being implemented should be source-specific and moment-specific during patient care (48).

3.4.1 Sources of organisms associated with hospital acquired infection

Identification of sources of organisms associated with HAIs in veterinary settings is critical to reducing the risk of transmission to patients and humans. Therefore, it is not surprising that most studies have largely focused on the hospital environment and commonly used instruments as potential reservoirs for organisms associated with HAIs (24,27,34,36,39). Furthermore, there are ongoing epidemiological studies to understand the relationship between environmental cleanliness and the risk of transmission of HAIs in veterinary settings (4).

The intensive care unit (ICU), surgical ward, in-house laboratory, and consultation rooms were the most important environmental sources of bacteria associated with HAIs in veterinary hospitals (15,18,25,31,37). Furthermore, environmental surfaces with human contact tend to have higher contamination levels compared to those without human contact (15,18,35,37). Suggesting that humans may play a major role in the transmission of these organisms within the hospital environment. This is further emphasised by studies that have isolated similar pathogens strains from the environment and hands of HCWs (23,43,47). Therefore, HCWs in veterinary hospitals must be trained on hand hygiene compliance to reduce the risk of transmission of HAI organisms.

Inanimate objects served as sources of HAI organisms and facilitated transmission between animal patients, the hospital environment, and humans (27). Inanimate objects such as clippers,

personnel clothing (27,34), cell phones (36), stethoscopes (34), and weighing scales (34) were reported to be contaminated with bacteria associated with HAIs. Therefore, the development and implementation of cleaning and disinfection protocols to prevent transmission is needed (2). In addition, all surgical material, instruments, and other fomites which increase the possibility of transmission of these organisms must be sterilised before use (31).

3.4.2 Bacterial isolates associated with hospital-acquired infections

3.4.2.1 Methicillin-resistant Staphylococcus aureus (MRSA)

Methicillin-resistant *Staphylococcus aureus* was among the most common organism associated with HAIs in this study (25,32). Furthermore, strains similar to those reported in humans were reported in this study (17,22,41). For example, Loeffler et al (22) in the UK identified MRSA clones (CC22 and CC30) among humans working with or in close proximity to animals suggesting transmission between animals and humans is precise (32). Studies also show that an unhygienic environment is a major source of MRSA (18,35,39) and implementing effective infection prevention and control (25,31,32,35,40,49) and screening animals before hospitalisation will reduce the spread of MRSA in veterinary hospitals. This is likely to reduce costs associated with increased length of hospital stay (17,18,22,31,41).

Most MRSA isolates in this study were resistant to β-lactam, 2nd generation cephalosporins, lincosamides, and aminoglycosides. While one study reported intermediate sensitivity to vancomycin among MRSA isolates (31). The presence of vancomycin resistance is concerning as it is the last resort for the treatment of MRSA in humans. Similarly, the presence of β-lactam resistance among staphylococci facilitated by the *mec*A gene (18,30–32,35,45) is likely to contribute to resistance to other antimicrobials with a β-lactam ring (35,37,40,41). Therefore, the implementation and constant review of infection control protocols are needed to help reduce the risk of the transfer of resistance genes to other organisms (40,50–52). Without these interventions, patient care and treatment will likely be negatively impacted (31,35).

3.4.2.2 Methicillin-resistant Staphylococcus pseudintermedius (MRSP)

Methicillin-resistant *Staphylococcus pseudintermedius* like MRSA has emerged as a leading cause of opportunistic infections in companion animals (27,37). The organism has been reported in

asymptomatic animals, implant-associated surgical sites (36), inanimate objects (27,36,40), and in the environment within the veterinary hospital (39). Therefore, colonized, and contaminated areas remain potential sources of hospital-acquired infections (27).

Of concern is that MRSP is highly resistant to antimicrobials commonly used for the treatment of *S. pseudintermedius* infections (53–55). These organisms have been isolated from the environment and hands of HCWs (37), which is concerning as it limits treatment options. Similar to MRSA, MRSP can acquire the *mec*A gene (37). Shoen *et al* (40) showed coagulase positive *S. pseudintermedius* commonly isolated from the skin of dogs can acquire the *mec*A gene from a coagulase-negative *S. epidermidis* commonly found in humans.

The zoonotic cases associated with MRSP are not common (27). However, an MRSA spa type 18/t338 from animal-related fomites has been reported in humans (36). The rise in the number of MRSP cases between dogs, pet owners, and veterinary staff is concerning, therefore, effective hand hygiene should be performed before and after contact with the patient, as well as after contact with potentially contaminated environmental sites within veterinary hospitals.

3.4.2.3 Enterococcus species

Enterococcus species are commensal of the gut flora of cats and dogs (3,38). However, they are also opportunistic pathogens (3). In recent years, *Enterococcus* species have emerged as causes of HAIs in veterinary medicine associated with urinary tract infections (UTIs) (56). The transmission is mainly due to faecal contaminated inanimate objects or environmental surfaces (24). These organisms can survive in a hospital environment for a long period. Furthermore, they can survive high temperatures and disinfectants such as chlorine and alcohol (37).

Enterococcus faecium and *E. faecalis* are the most predominant species reported in dogs (38), hospital environments and in hands of HCWs (37). Of the two species, *E. faecalis* is the predominant enterococci. Multidrug-resistant enterococci have also been reported as a commensal and pathogenic organism (3,37,38). The presence of MDR among *Enterococcus* species has largely been attributed to overuse and misuse of antimicrobials (37,38). It is also possible that some may have acquired resistance through other mechanisms including genetic transfer or mutation (38). For example, resistance to erythromycin has been associated with the methylation of the ribosomal

target site of these antibiotics (37,57). Nonetheless, the presence of MDR enterococci is likely to impact patient care in veterinary hospitals (37).

Of concern is the emergence of vancomycin-resistant *E. faecium* (37) which is an important antimicrobial in the treatment of enterococci infections (38,57) and is mediated by the presence of *van*A genes. These genes are important as they confer multidrug resistance and may be transmitted to other bacterial species such as *Staphylococcus* spp. and create even bigger problems in the treatment of HAIs (37). Furthermore, these gene carrying bacteria can also be transferred from animals to humans (3,38).

3.4.2.4 Clostridium difficile

Clostridium difficile is found in the hospital environmental and it is difficult to eradicate (15). Both humans and animals are asymptomatically carriers of the organism. In humans, its presence has been attributed to the overuse of antimicrobials. However, in veterinary medicine there is limited information about the organism. Therefore, future studies should look at whether the overuse of antimicrobials could be a driver of *C. difficile* in veterinary settings (15). The ability of the pathogen to survive harsh environmental conditions and resistance to most disinfectants makes it a suitable indicator of the effective IPC measures (15). Therefore, it is possible that this organism can also be used as an indicator of effective infection prevention and control in veterinary hospitals.

3.4.2.5 Acinetobacter baumannii

Acinetobacter baumannii causes life-threatening infections in both humans and animals. This organism has been reported in UTIs, pyothorax, upper airway obstruction, bloodstream infection, and wound infections in animals (34). In infected animals, it is associated with increased morbidity and prolonged length of hospital stay (58). *Acinetobacter baumannii* survives on dry surfaces (24,34,59). Therefore, commonly used equipment, bed rails, cages, and examination tables could serve as reservoirs for *A. baumannii.* Most *A. baumannii* are multiple drug resistant with a high prevalence of resistance towards cephalexin, enrofloxacin, amoxicillin-clavulanic acid, sulphamethoxazole-trimethoprim, and tetracycline (34). Resistance to the above antimicrobials is concerning as these antimicrobials are commonly used for the treatment of bacterial infections in

small animal medicine (58). In addition, the *bla*OXA-51 gene reported in an *A. baumannii* isolate from pigs has also been reported in humans (57).

3.4.2.6 Escherichia coli

Escherichia coli is commonly reported in UTIs and bloodstream infections (15,26,34,60). The bacterium spreads from patient to patient via faecal contaminated hands of HCWs and shared equipment (26). Given, environmental surfaces could potentially be a reservoir of *E. coli*, measures to minimise faecal contamination in companion animal hospitals including cleaning and disinfection of the hospital environment should be implemented. Moreover, Sanchez et al (26) shows the transfer of *E. coli* isolates with similar antimicrobial resistance patterns between two different animals admitted to the same ICU.

In the current study, *E. coli* isolates exhibited resistance towards cephalosporins and β-lactams including amoxycillin-clavulanic acid. This broad-spectrum antimicrobial resistance among *E. coli* is attributed to the presence of *amp*C like gene, bla_{CMY2} (15,26), which has been identified to be of public health concern (15). Another study reported resistance among *E. coli* isolates to chloramphenicol mainly due to the presence of *cmlA* homologue *flo* among gram-negative bacteria (26). The presence of these genes has also been linked to the development of resistance to other commonly used antibiotics such as gentamycin, spectinomycin, and sulfadimethoxine (26,34,46). Considering this resistance, strict guidelines should be implemented on the prudent use of antimicrobials in veterinary medicine in South Africa.

3.4.2.7 Salmonella species

Although most animals are asymptomatic carriers of *Salmonella* spp., they shed the bacterium in high quantities through their faeces (43). Asymptomatic animals have been linked to *Salmonella* outbreaks in equine veterinary medicine (42,43). Furthermore, infections associated with *Salmonella* species have also been reported in bovine and companion animals (36,42). In affected animals, the disease is characterized by high morbidity and mortality. Furthermore, the potential spread and zoonotic infection in veterinary hospitals often result in the closure of facilities with a loss of income for the hospital (33,42). Managing transmission in the veterinary settings remains a challenge as *Salmonella* can persist in the environment for a long time. In addition, personnel

working in close contact with infected animals (42). Rodents and contaminated feed could also be a source (42,43). Therefore, biosecurity measures must be intensified in veterinary hospitals to reduce the risk of transmission. Additionally, education programs can also be developed targeting specific aspects of hygiene, movement control, and cleanliness of equipment.

Salmonella isolates were resistant to ceftiofur, gentamycin, amoxicillin, ampicillin, streptomycin, and trimethoprim/ sulfadiazine (42,43). One study reported the presence of the cephalomycinase gene, bla_{cmy-2} (43) which has been associated with cephalosporin resistance among *Salmonella* species. This gene has also been reported to mediate resistance to amoxicillin, amoxicillin-clavulanic acid, cephalothin, cefoxitin, ceftiofur, and ceftriaxone (43).

3.5 Conclusion

Organisms associated with hospital-acquired and zoonotic diseases were reported from clinical cases, environmental surfaces, and items used in veterinary service. The hospital environment with human contact was the most reported source of organisms associated with HAIs. These results suggest that humans play a crucial role in the transmission of bacteria associated with HAIs in veterinary hospitals.

Among the organisms reported, MRSA *and* MRSP were the most reported HAI organisms in veterinary facilities. Other organisms identified include *E. coli, C. difficile, A. baumannii, Salmonella* spp., and *Enterococcus* species. Some of these isolates reported in veterinary settings share similar clonal lineage to those reported in humans. Some organisms exhibit a high prevalence of antimicrobial resistance and contain genes known to be associated with antibiotic resistance.

These results suggest that strict infection prevention and control practices must be in place, monitored and modified when necessary to curb the occurrence and transmission of organisms associated with HAIs in veterinary hospitals. In addition, continuous surveillance of HAI organisms and their antimicrobial resistance patterns in veterinary hospitals should be emphasized. Further research needs to be done on *C. difficile* as a potential indicator of effective infection prevention and control practices in veterinary facilities.

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Chapter 4

Antimicrobial resistance patterns of *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated from dogs presented at a veterinary academic hospital in South Africa

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My contribution to the paper includes study design, data analysis, interpretation of results, writing of manuscript as well as extensive editing of the manuscript.

Abstract

Background: *Acinetobacter baumannii* and *Klebsiella pneumoniae* are opportunistic bacterial pathogens responsible for hospital-acquired infections (HAIs) in veterinary medicine. Infection with these bacteria always requires urgent antimicrobial therapy. However, there is no evidence of studies that have investigated the antimicrobial drug resistance profile of these organisms in a veterinary setting in South Africa. This study investigated the antimicrobial resistance patterns of *A. baumannii* and *K. pneumoniae* from clinical specimens obtained from dogs presented at a veterinary academic hospital. The findings of the present study contribute to an improved understanding of the antimicrobial resistance profile of these bacteria in veterinary medicine.

Methods: Retrospective data of clinical samples from dogs that were positive for *A. baumannii* and *K. pneumoniae* between 2007 and 2013 were used in this study. Antimicrobial susceptibility for the isolates was determined using the disk diffusion method following the Clinical and Laboratory Standards Institute guidelines (CLSI). The *A. baumannii* isolates were subjected to a panel of 20 antibiotics, while *K. pneumoniae* isolates were subjected to a panel of 22 antibiotics. Data were analysed using descriptive statistics and presented using tables and figures.

Results: Twenty (n=20) *A. baumannii* isolates were isolated from bronchoalveolar lavage, foreign objects, bone, urine, skin, blood, ear, nasal, and oral cavity. Almost all *A. baumannii* (95%, 19/20) isolates were resistant to at least one antibiotic, and 60% (12/20) were multidrug-resistant (MDR). *Klebsiella pneumoniae* (n=56) was isolated from urine, foreign objects, abscesses, ears, eyes, tracheal aspirations, bronchoalveolar lavages, eyes, abdominal aspirates, anal glands, bones, intestinal and lung biopsies. All *K. pneumoniae* (100%, 56/56) isolates were resistant to at least one antibiotic, and 98% (55/56) were MDR.

Conclusion: Both *A. baumannii* and *K. pneumoniae* were isolated in various clinical tissue samples and exhibited a high prevalence of resistance to multiple antibiotics. In addition, these bacteria

exhibited a high prevalence of resistance to β-lactam compared to other classes of antibiotics, which is likely to impact treatment options and patient prognosis.

4.1 Introduction

Acinetobacter baumannii and *klebsiella pneumoniae* belong to the group of bacteria termed 'ESKAPE' pathogens, and they are responsible for outbreaks in clinical settings across the globe (1). This ESKAPE group of bacteria is known to escape the biocidal action of antimicrobials and is associated with increased mortality and healthcare costs in both human and animal medicine (1). In addition, these bacterial species are among the pathogens for which urgent antimicrobial therapy is required due to their tendency to exhibit a high prevalence of multidrug resistance (MDR) (1,2).

Acinetobacter baumannii is an opportunistic pathogen that usually affects immunocompromised patients (3). It is a non-motile, aerobic, oxidase-negative, non-fermentative coccobacilli gram-negative bacterium (4). It is ubiquitous and has been isolated from drinking water, food, and soil (4,5). *Acinetobacter baumannii* can form biofilms that enable it to survive for long periods on dry surfaces and medical devices in the ICU. As a result, surfaces of inanimate objects in hospitals can be a source of infection for patients (4,6). In humans, *A. baumannii* has been isolated from clinical infections such as pneumonia, bloodstream infections, skin and soft tissue infections, urinary tract infections (UTIs), and meningitis, while it has been isolated in dogs from UTIs, bloodstream infections, and wound infections (4,5). *Acinetobacter baumannii* associated with hospital-acquired infections (HAIs) has been shown to be multidrug-resistant (MDR) and with a high prevalence of resistance to the β-lactam and cephalosporin groups of antibiotics (4). Among the reasons for the high prevalence of resistance to these groups is the intrinsic resistance associated with the interplay between the outer membrane providing protection, active efflux pump systems, and the low-quantity expression of small-aperture outer membrane porins (6). *Klebsiella pneumoniae* is a facultative, anaerobic gram-negative bacterium belonging to the *Enterobacteriaceae* family. It is an intestinal commensal; however, it has been reported in gastrointestinal (GIT) diseases, UTIs, pneumonia, bacteraemia, pyogenic liver abscesses, and burn

and wound infections in both humans and animals (6,7). Together with *Escherichia coli,* these bacteria are among the most prevalent organisms in hospital and community settings (6,8). *Klebsiella pneumoniae* is an opportunistic pathogen in young, old, and immunocompromised humans (6). It is an important cause of hospital-acquired wound infections and UTIs in humans (7). In animals, the bacterium has been reported in clinical mastitis, pneumonia, septicaemia, bacteraemia, UTIs, and polyarthritis (7). *Klebsiella pneumoniae* exhibits a high prevalence of resistance to multiple antibiotics (6,7,9). It acquires and disseminates resistant genes, including those encoding for the extended spectrum β- lactamases (ESBLs), resulting in resistance to β-lactam antibiotics, including penicillin, cephalosporins, and the monobactam aztreonam (6,9), therefore, limiting treatment options (8,9).

In South Africa, studies of ESKAPE pathogens have been well-documented in human medicine (2,10,11). However, studies investigating antimicrobial drug resistance among the ESKAPE group of pathogens in veterinary medicine are limited. This study investigated the antimicrobial resistance patterns of *K. pneumoniae* and *A. baumannii* isolated from clinical samples of dogs presented at a veterinary teaching hospital. The findings of this study will contribute to a better understanding of antibiotic resistance among *K. pneumoniae* and *A. baumannii* isolates of veterinary origin. In addition, it is envisaged that information generated from this study will be used to guide the treatment of *K. pneumoniae* and *A. baumannii* infections and improve treatment outcomes in a veterinary setting (6).

4.2 Materials and methods

4.2.1 Study area

This study was conducted at a veterinary academic hospital in Pretoria, South Africa. The hospital provides clinical services for companion, livestock, and wildlife animals. In addition, the hospital serves as a referral for internal medicine and surgical cases for clients in and around Pretoria. The bacteriology laboratory in the Department of Veterinary Tropical Diseases that cultured the isolates provides a service to the veterinary academic hospital for routine clinical diagnosis of suspected infectious diseases.

4.2.2 Data source

Retrospective data records of dog clinical samples were submitted to the bacteriology laboratory between January 2007 and December 2013. For each isolate, the following information was extracted from the paper records: the patient's unique number, specimen type, date of sample collection, organ system, and antimicrobial susceptibility test results of the isolates. The data were then entered and stored in an electronic database for analysis.

Bacterial isolates and antimicrobial susceptibility testing

All the submitted clinical samples were cultured to isolate *A. baumannii* and *K. pneumoniae* using standard bacteriological methods described by Ricketts et al. (12). Antimicrobial susceptibility testing was performed using the disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) (13) guidelines to conduct antimicrobial susceptibility testing.

Acinetobacter baumannii isolates were subjected to a panel of 20 antibiotics: amikacin (30 µg), ampicillin (10µg), carbenicillin (100µg), ceftazidime (30µg), cephalothin (30µg), chloramphenicol (30µg), enrofloxacin (5µg), gentamicin (10µg), imipenem (10µg), kanamycin (30µg), lincomycin (10µg), lincomycin-spectinomycin (100µg), orbifloxacin (5µg), oxytetracycline (30µg), penicillin-G (10µg), piperacillin (100µg), trimethoprim-sulphamethoxazole (25µg), amoxicillin/clavulanic acid (20/10µg), tobramycin (10µg), and tylosin (15µg) (Oxoid Ltd., Cambridge, UK).

Klebsiella pneumoniae isolates were subjected to a panel of 22 antibiotics: amikacin (30µg), ampicillin (10µg), carbenicillin (100µg), ceftazidime (30µg), cephalothin (30µg), chloramphenicol (30µg), enrofloxacin (5µg), erythromycin (15µg), gentamicin (1µg), imipenem (10µg), kanamycin (30µg), lincomycin (10µg), lincomycin-spectinomycin (100µg), orbifloxacin (5µg), oxytetracycline (3µg), penicillin G (10µg), piperacillin (100µg), rifampin (30µg), trimethoprim-sulphamethoxazole (25µg), amoxicillin/clavulanic acid (20/10µg), tobramycin (10µg), and tylosin (15µg) (Oxoid Ltd., Cambridge, UK).

The results of the antibiograms were classified as intermediate, susceptible, or resistant, following the CLSI guidelines (13). For the purposes of this study, resistance to at least one antibiotic was classified as AMR. Multidrug resistance was defined as resistance to at least one antibiotic in three or more antibiotics categories (14).

Antimicrobials to which the bacteria have an inherent resistance were excluded from MDR analysis. For example, *Klebsiella pneumoniae* is known to be inherently resistant to ampicillin, carbenicillin, and erythromycin. Therefore, these groups of antibiotics were excluded from the MDR analysis. Since *A. baumannii* is inherently resistant to penicillins and lincosamides, these two groups were excluded from the analysis to determine the prevalence of MDR. In addition, antibiotics were excluded from the MDR analysis if all isolates were not tested to determine their susceptibility to these antibiotics. Based on this, imipenem, tobramycin, rifamycin, and ceftazidime were excluded from the analysis to determine MDR isolates for *K. pneumoniae*, and imipenem, tobramycin, and ceftazidime were excluded from the analysis to determine MDR isolates for *A. baumannii.*

4.2.3 Data management and analysis

The dataset was assessed for duplicates and missing information, such as the lack of antibiogram results. Some isolates had missing information, but there were no duplicates in the dataset. Isolates from specimens such as endotracheal (ET) tubes, screws, pins, wires, catheter tips, nails, and plates were classified as “foreign objects”, while specimens such as lung, liver, spleen, lymph node,

heart, and kidney were reclassified as “organ pool”. Crude percentages of isolates of *A. baumannii and K. pneumoniae* that were AMR and MDR were computed and presented as figures and tables. All statistical analyses were performed using the Statistical Analysis System (SAS).

4.2.4 Ethical consideration

Written consent granting the academic teaching hospital permission to use information obtained from dogs presented at the hospital for teaching and research purposes was obtained from the owners of the dogs. In addition, this study followed all ethical standards for research without direct contact with human or animal subjects. Ethical clearance was also obtained from the University of Pretoria’s Faculty of Veterinary Science Research Ethics Committee, Faculty of Humanities Research Ethics Committee (Project number: REC009-21) and Faculty of Health Sciences Research Ethics Committee (Reference No: 187/2022).

4.3 Results

4.3.1 Acinetobacter baumannii

A total of 20 *A. baumannii* were isolated over the study period with six (n=6; 30%) from bronchoalveolar lavage and three (n=3; 15%) from foreign objects. *Acinetobacter baumanni* was also isolated from various samples/tissues such as bone, urine, skin, blood, ear, nasal, and oral cavity (**Figure 4.1**).

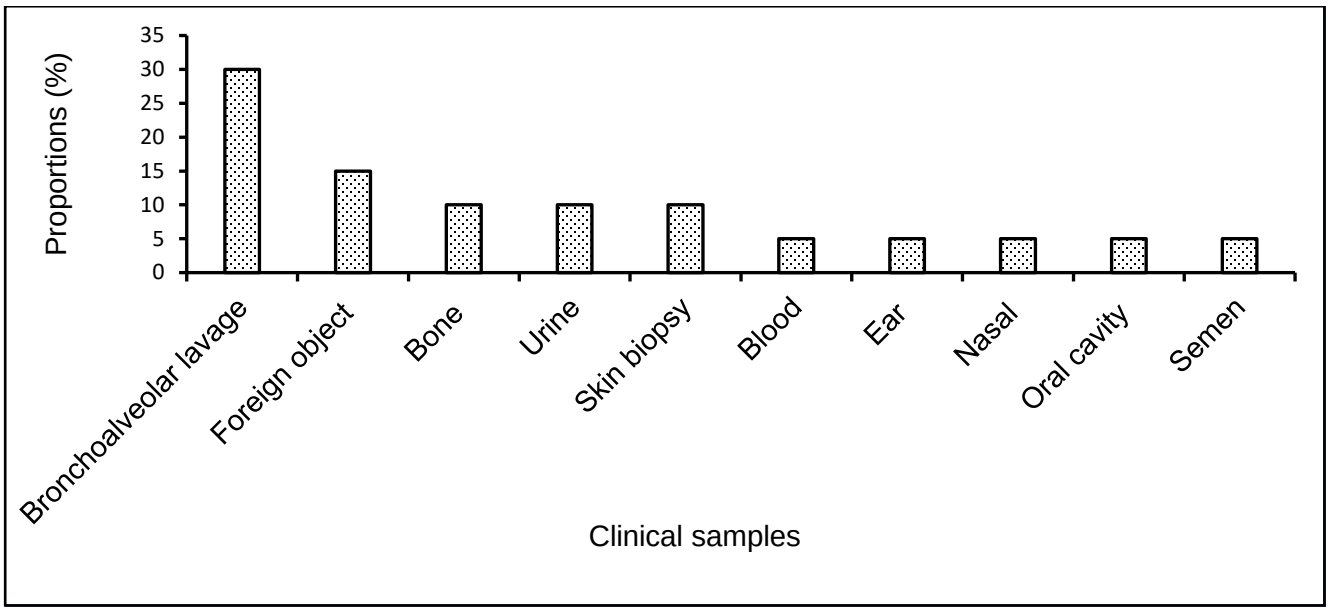

Figure 4. 1: Distribution of *Acinetobacter baumannii* in the various dog clinical samples tested by the Bacteriology Laboratory that services that Veterinary Academic Teaching Hospital, 2007 - 2013.

4.3.1.1 Antimicrobial resistance and multidrug resistance

Nineteen isolates were resistant to at least one antibiotic (95%, 19/20) with the majority of *A. baumannii* isolates showing resistance to penicillin-G (85%) and ampicillin (65%). Forty-five per cent (45%, 9/20) of the isolates were resistant to amoxicillin/clavulanic acid. All five isolates (100%) tested were resistant to carbenicillin, piperacillin, and ceftazidime. A high prevalence of resistance was recorded towards lincomycin (95%), tylosin (68%), chloramphenicol (60%), lincomycin-spectinomycin (60%), and cephalexin (60%). A low prevalence of resistance among the *A. baumannii* was reported for aminoglycosides, except for tobramycin. Similarly, low resistance was observed against fluoroquinolones, tetracycline, and potentiated sulfonamides. One out of four (1/4, 25%) isolates was resistant to imipenem **(Table 4.1)**.

Sixty percent (60%, 12/20) of *A. baumannii* isolates were MDR. A high proportion of isolates exhibited resistance to cephalothin (92%), followed by chloramphenicol, trimethoprim-sulphamethoxazole, enrofloxacin, amoxicillin/clavulanic acid, and kanamycin, to which 75% of the

isolates were resistant **(Table 4.1).** Three (n=3) MDR-*A. baumannii* isolates were resistant to 10 antimicrobials, two (n=2) to nine antimicrobials, and one (n=1) to 8 antimicrobials (**Table 4.2**).

Table 4. 1: Antimicrobial resistance and multidrug resistance profile of *Acinetobacter baumannii* isolated from dog clinical samples tested at a veterinary academic hospital, South Africa.

Antimicrobial category	Resistant	
	Isolates %(n)	MDR isolates %(n)
Macrolides		
Tylosine	68 (13/19)	-
β- lactams		
Penicillins		
Ampicillin	65 (13/20)	-
Carbenicillin	100 (5/5)	-
Penicillin-G	85 (17/20)	-
Piperacillin	100 (5/5)	-
Cephalosporins		
Ceftazidime	100 (5/5)	-
Cephalothin/lexin	60 (12/20)	92 (11/12)
Combination		
amoxicillin/clavulanic acid	45 (9/20)	75 (9/12)
Carbapenem		
Imipenem	25 (1/4)	-
Aminoglycosides		
Amikacin	30 (6/20)	50 (6/12)
Gentamicin	20 (4/20)	33 (4/12)
Kanamycin	47 (9/19)	75 (9/12)
Tobramycin	80 (4/5)	-
Lincosamides		-
Lincomycin	95 (19/20)	-
lincomycin-spectinomycin	60 (12/20)	-
Potentiated sulfas		
trimethoprim-sulphamethoxazole	45 (9/20)	75 (9/12)
Fluoroquinolones		
Orbifloxacin	40 (8/20)	67 (8/12)
Enrofloxacin	45 (9/20)	75 (9/12)
Tetracycline		
Oxytetracycline	35 (7/20)	50 (6/12)
Amphenicols		
Chloramphenicol	60 (9/15)	75 (9/12)

Table 4. 2: Antibiotics resistance patterns of *Acinetobacter baumannii* isolated from dog clinical samples presented in a veterinary academic hospital in South Africa.

Patterns	Number
AMI_CEP_CHL_OXY_ENR_GEN_KAN_ORB_SUL_SYN	3
AMI_CEP_CHL_OXY_ENR_KAN_ORB_SUL_SYN	2
CEP_CHL_SYN	2
CEP_CHL_ENR_GEN_KAN_ORB_SUL_SYN	1
CEP_OXY_ENR_ORB_SUL_SYN_	1
CEP_CHL_ENR_KAN_ORB_SUL	1
AMI_CEP_KAN_	1
ENR_KAN_SUL	1
Total	**12**

AMI=Amikacin, CEP= Cephalothin/lexin, CHL=Chloramphenicol, OXY= Oxytetracycline, ENR=Enrofloxacin, GEN=Gentamicin, KAN=Kanamycin, ORB=Orbifloxacin, SUL= trimethoprim-sulphamethoxazole SYN=Synulox

4.3.2 Klebsiella pneumonia

A total of 56 *Klebsiella pneumoniae* isolates were recorded. Of these, 39% (22/56) were isolated from urine samples, followed by 9% (5/56) from foreign objects. Very low proportions were isolated from abscesses, ears, eyes, transtracheal aspirations, bronchoalveolar lavage, abdominal aspirates, anal glands, bones, intestinal, and lung biopsies **(Figure 4.2).**

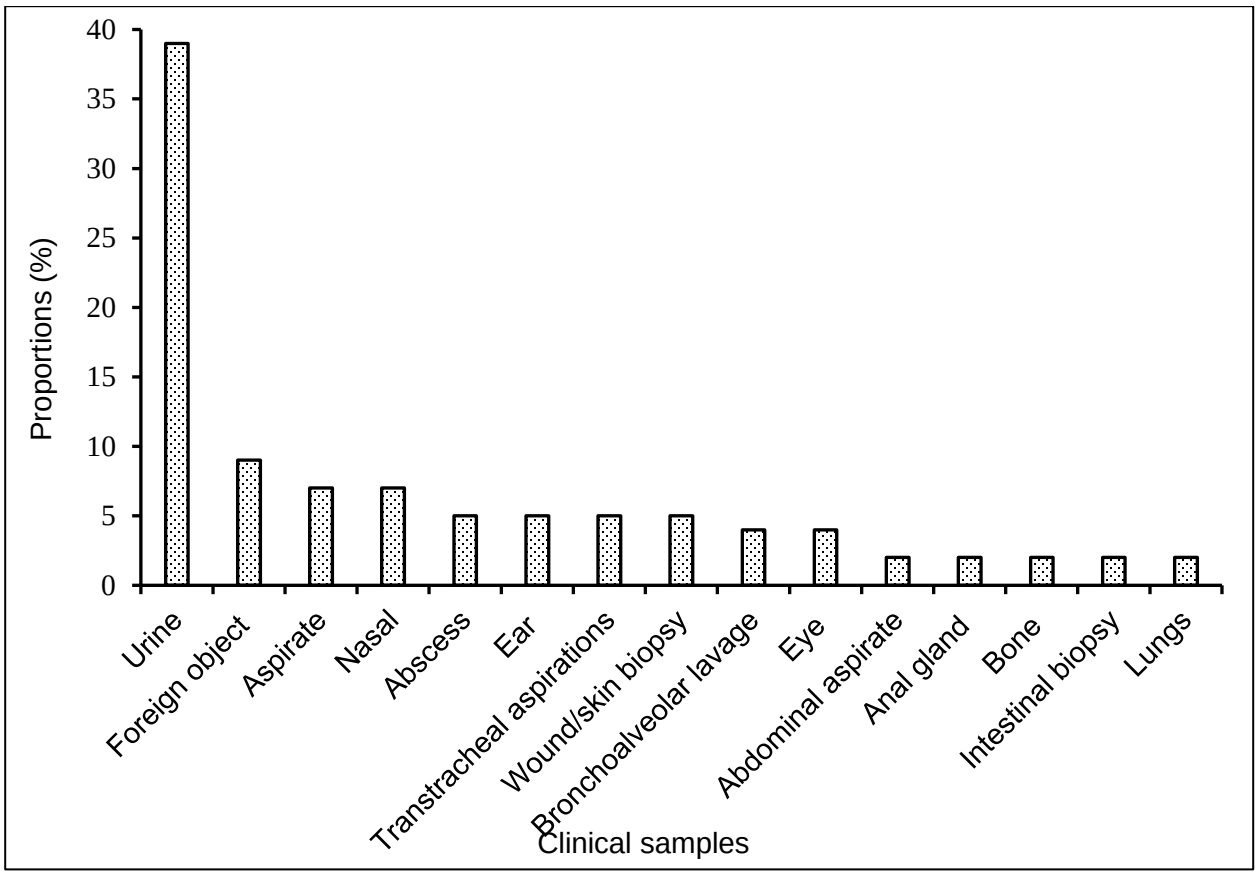

Figure 4. 2: Distribution of *Klebsiella pneumoniae* in the various dog clinical samples tested by the bacteriology laboratory at the faculty of veterinary science between 2007 and 2013.

4.3.2.1 Antimicrobial resistance and multidrug resistance

All the *K. pneumoniae* isolates were resistant to penicillin-G, ampicillin, carbenicillin, piperacillin, ceftazidime, and lincomycin. Sixty-four percent (35/56) of the isolates were resistant to cephalexin and 60% to amoxicillin/clavulanic acid. None of the isolates tested were resistant to imipenem**.** *Klebsiella pneumoniae* exhibited a high prevalence of resistance to antibiotics belonging to aminoglycosides, tobramycin (88%), and kanamycin (63%). Ninety-four percent (94%) of the isolates were resistant to tylosin and 70% to oxytetracycline. One (n=1) *K. pneumoniae* isolate tested showed resistance to both erythromycin and rifampin. Ninety-eight percent (98%) of resistant *K. pneumoniae* isolates were MDR, with most being resistant to penicillin-G (100%), and tylosin tartrate (93%) **(Table 4.3).** The most common resistance pattern among the MDR *K. pneumoniae* isolates included the combination of licomycin - penicillin-G – tylosin (**Table 4.4**).

Table 4. 3: Antimicrobial resistance and multidrug resistance profile of *Klebsiella pneumoniae* isolated from dog clinical samples tested at a veterinary academic hospital, South Africa.

Antimicrobial category	Resistant	
	Isolates %(n)	MDR isolates %(n)
Macrolides		
Erythromycin	100 (1/1)	-
Tylosine	94 (51/54)	93 (51/55)
β - lactams		
Penicillins		
Ampicillin	100 (56/56)	-
Carbenicillin	100 (7/7)	-
Penicillin G	100 (56/56)	100 (55/55)
Piperacillin	100 (8/8)	-
Cephalosporins		
Ceftazidime	100 (8/8)	-
Cephalothin/lexin	64 (35/55)	64 (35/55)
Combination		
amoxicillin/clavulanic acid	60 (33/55)	60 (33/55)
Carbapenem		
Imipenem	0 (0/8)	-
Aminoglycosides		
Amikacin	48 (27/56)	49 (27/55)
Gentamicin	41 (23/56)	42 (23/55)
Kanamycin	63 (33/52)	60 (33/55)
Tobramycin	88 (7/8)	-
Lincosamides		
Lincomycin	100 (54/54)	-
lincomycin-spectinomycin	72 (38/53)	
Rifamycin		
Rifampin	100 (1/1)	-
Potentiated-sulfas		
trimethoprim-sulphamethoxazole	36 (20/56)	36 (20/55)
Fluoroquinolones		
Enrofloxacin	39 (22/56)	38 (21/55)
Orbifloxacin	49 (26/53)	47 (26/55)
Amphenicols		
Chloramphenicol	41 (19/46)	35 (19/55)
Tetracycline		
Oxytetracycline	70 (39/56)	71 (39/55)

Table 4. 4: Antibiotics resistance patterns of *Klebsiella pneumoniae* isolated from dog clinical samples presented in a veterinary academic hospital in South Africa.

PATTERN	Number
LIN_PNG_TYL	7
AMI_CEP_CHL_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_SYN_TYL	3
AMI_CEP_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_SUL_SYN_TYL	2
AMI_CEF_CEP_CHL_OXY_GEN_KAN_LIN_LCS_ORB_PNG_PIP_SUL_SYN_TOB_TYL	2
AMI_CEF_CEP_CHL_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_PIP_SUL_SYN_TOB_TYL	2
AMI_CEP_CHL_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_SUL_SYN_TYL	2
AMI_CEP_OXY_GEN_KAN_LIN_LCS_PNG_SYN_TYL	2
CEP_CHL_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_SUL_SYN_TYL	1
OXY_LIN_LCS_PNG_SUL_TYL	1
LIN_LCS_PNG_TYL	1
AMI_CEP_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_SYN_TYL	1
OXY_LIN_PNG_SYN_TYL	1
AMI_CEP_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_TYL	1
AMI_CEP_CHL_OXY_GEN_KAN_LIN_PNG_SYN_TYL	1
AMI_CEP_OXY_GEN_KAN_LIN_LCS_ORB_PNG	1
AMI_CEP_CHL_OXY_KAN_LIN_LCS_ORB_PNG_SUL_SYN_TYL	1
CEP_CHL_OXY_KAN_LIN_LCS_ORB_PNG_SYN_TYL	1
OXY_LIN_LCS_PNG_TYL	1
CEP_ENR_KAN_LIN_ORB_PNG_SYN_TYL	1
AMI_CEP_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_SUL_SYN_TOB_TYL	1
CEP_OXY_ENR_KAN_LIN_LCS_ORB_PNG_SYN_TYL	1
CEP_CHL_OXY_ENR_LIN_LCS_ORB_PNG_SUL_SYN_TYL	1
CEP_OXY_LIN_LCS_PNG_TYL	1
ENR_LIN_PNG_TYL	1
CEP_KAN_LIN_LCS_PNG_SUL_TYL	1
AMI_CEP_OXY_LIN_LCS_PNG_SUL_TYL	1
CEP_OXY_LIN_LCS_PNG_SUL_SYN_TYL	1
AMI_CEP_PNG_TYL	1
CEP_OXY_LIN_PNG_SYN_TYL	1
AMI_CHL_OXY_ENR_GEN_LIN_PNG_SUL_TYL	1
KAN_LIN_LCS_PNG_SYN_TYL	1
AMI_OXY_KAN_LIN_LCS_PNG_TYL	1
LIN_PNG_SYN	1
AMI_CEF_CEP_CHL_OXY_ENR_GEN_KAN_LIN_LCS_ORB_PNG_PIP_SUL_SYN_TOB_TYL_	1
OXY_KAN_LIN_LCS_PNG_TYL	1
AMI_CEF_CEP_KAN_LIN_LCS_ORB_PNG_PIP_SYN_TYL	1
OXY_LIN_LCS_PNG_SYN_TYL	1
AMI_CEP_CHL_OXY_KAN_LIN_ORB_PNG_SYN_TYL	1
OXY_LIN_PNG	1

AMK_GEN_KAN_LIN_LNC_PNG	1
CEF_CEP_CHL_OXY_ENR_ERT_GEN_KAN_LIN_LCS_ORB_PNG_PIP_RIF_SUL_SYN_TOB_TYL	1
CEF_CEP_CHL_OXY_ENR_KAN_LIN_LCS_ORB_PNG_PIP_SUL_SYN_TOB_TYL	1
Total	**55**

AMI=Amikacin, CEF= Ceftazidine, CEP= Cephalothin/lexin, CHL=Chloramphenicol, OXY=Oxytetracycline, Enr=Enrofloxacin, ERT= Erythromycin, GEN= Gentamicin, KAN=Kanamycin, LIN= Lincomycin, LCS= Lincospectin, ORB=Orbifloxacin, PNG= Penicillin-G, PIP-Piperacin, RIF=Rifampin, SUL= Trimethoprim-sulphamethoxazole , SYN=Synulox, TOB= Tobramycin, TYL= Tylosine Tartrate

4.4 Discussion

This study investigated the antimicrobial resistance patterns of *A. baumannii* and *K. pneumoniae* isolated from dog cases presented at a veterinary hospital in South Africa. Similar to findings reported by other studies, *A. baumannii* and *K. pneumonia* were isolated in various clinical samples. This confirms past research findings that reported these organisms as associated with various clinical infections in dogs (5,7,15–18). Even more concerning is that these organisms have been associated with HAIs can disseminate resistance genes to other bacteria (19,20). Cleaning and disinfection of the environment have proven effective in reducing the burden of these organisms in the environment (21). However, these organisms can persist in a dry environment and continue to be a source of infection to susceptible patients (15,21,22). Therefore, careful monitoring of dogs admitted to the veterinary hospital through routine surveillance is important to prevent the transmission of these pathogens between patients.

4.4.1 Antibiotic resistance patterns of Acinetobacter baumannii

Antibiotic resistance among *A. baumannii* isolates is increasing and is associated with increased morbidity, mortality, and treatment costs in the intensive care unit (ICU) (23). In this study, a high prevalence of resistance among *A. baumannii* to β-lactam antimicrobials, including penicillin, cephalosporins, and amoxicillin/clavulanic acids, was observed. This is concerning as these antimicrobials are commonly used in small animal practices to treat uncomplicated infections (4). The high prevalence of resistance observed in this study is consistent with that reported in veterinary studies done in the United States of America (24), Switzerland (25), and Malaysia (4). This is attributed to the wide array of antimicrobial-inactivating enzymes, including β-lactamases, that confer resistance to the β-lactam groups of antimicrobials (19,25,26) and the overexpression of the chromosomally encoded *Amp*C cephalosporinases conferring resistance to broad-spectrum cephalosporins (26,27).

A low prevalence of resistance to imipenem among *A. baumannii* has been reported in a study by Pailhories (15). In the present study, only one (1/4) isolate was resistant to imipenem.

However, a larger sample size is needed to determine the carbapenem susceptibility profile of *A. baumannii,* considering it is the treatment of choice in humans (5).

Acinetobacter baumannii was resistant to trimethoprim-sulphamethoxazole, which is consistent with findings in other studies (28,29). This could be due to the overproduction or alteration in plasmid-mediated dihydrofolate reductase associated with trimethoprim resistance (30). Although *A. baumannii* exhibited resistance to trimethoprim-sulphamethoxazole, evidence suggests that it should be considered for uncomplicated infections (29,31,32).

Resistance to aminoglycosides among *A. baumannii* was generally not common in this study, with the exception of tobramycin. This was expected given that resistance to tobramycin among *A. baumannii* increased (33,34), mainly associated with the synthesis of aminoglycoside-modifying enzymes (AME) and efflux pump systems (33,35). This finding has significant public health implications, given that aminoglycosides are commonly used to treat *A. baumannii* infections. In view of this, trends in the susceptibility of these organisms should be monitored (26,33).

Fluoroquinolones are generally used to treat *A. baumannii* infections in small animals (4). In this study, a low prevalence of resistance to fluoroquinolones was observed. These organisms' resistance to fluoroquinolones could be due to the overuse of the antibiotics and is mediated by the efflux-mediated quinolones resistance (27,36–38). Therefore, care is needed to prevent misuse and overuse of fluoroquinolones to curb the development of resistance (39,40). A low prevalence of resistance to oxytetracycline was also observed in this study. This is encouraging because of the potential use of tetracyclines as monotherapy or in combination with other antimicrobials for the treatment of *A. baumannii* infections (28,41,42).

Forty-five percent (n=5; 45%) of *A. baumannii* isolates were MDR. However, a higher prevalence of *A. baumannii* (83.3%, 5/6) from environmental samples exhibiting MDR was reported by Ng et al (4) in a study conducted in Malaysia. The high prevalence of MDR-*A. baumannii* is not uncommon (43). Given this, available evidence suggests that the choices for treatment of MDR *A. baumannii* infections may include carbapenems, colistin and combination antimicrobials (4,26,33,44).

4.4.2 Antibiotic resistance patterns of Klebsiella pneumoniae

Similar to the findings by Haenni et al (45) in France and Lee et al (21) in South Korea, most *K. pneumoniae* isolates in this study were resistant to β-lactam antimicrobials. The β-lactam resistance among *K. pneumoniae* isolates is attributed to the production of the plasmid-mediated sulfhydryl variable (SHV-1) a penicillinase (9,18,45–47). On the other hand, none of the *K. pneumoniae* isolates in this study exhibited resistance to carbapenems. This is consistent with the findings by Haenni et al (45) in the study conducted in France. These findings suggest that carbapenem could be considered as part of the treatment option for *K. pneumoniae* (47,48).

The prevalence of resistance to aminoglycosides varied in this study. For example, low resistance was observed to amikacin and gentamycin (49,50), while high resistance was observed to tobramycin and kanamycin. The varying prevalence of resistance among aminoglycosides could be attributed to the different mechanisms of resistance. For example, resistance to amikacin and gentamycin is associated with the presence of aminoglycoside modifying enzymes (AME) and/or 16S ribosomal RNA methyltransferase (16S-RMTases) (3,49,51), whereas tobramycin resistance has been associated with the presence of AAC (6')-Ib(-like) protein and not AME or 16S-RMTase genes (52). Despite the nephrotoxicity of aminoglycosides (53), this group of antimicrobials have been used effectively in the treatment of *K. pneumoniae* infections in both human and veterinary medicine (3).

Consistent with findings from both human and animal studies (17,18,47), resistance to enrofloxacin and orbifloxacin among *K. pneumoniae* isolates was low in this study. Similar to other studies, resistance to trimethoprim-sulphamethoxazole was observed in this study (18,37). The low resistance in this study is encouraging, as trimethoprim-sulphamethoxazole is the drug of choice in the treatment of UTIs (18,54). In addition, trimethoprim-sulphamethoxazole is effective in the treatment of patients with carbapenemase-producing *K. pneumoniae* infections (55).

Almost all *K. pneumoniae* isolates in this study were MDR. This is not unusual, as antimicrobial resistance genes are frequently observed in this organism (18). What is of concern is that the role of companion animals as reservoirs for human infections associated with resistant *K.*

pneumoniae is not well described in the literature. Therefore, further studies are needed to investigate the transmission of resistant genes between humans and animals.

4.5 Limitations

The data used for the study was limited to one veterinary hospital and did not include other veterinary medical facilities. Since the hospital that provided the data is a referral hospital, it is possible that most isolates may have had previous exposure to antibiotics.

4.6 Conclusion

Acinetobacter baumannii and *K. pneumoniae* were identified from various clinical samples suggesting that they are important causes of infections in dogs and can infect various body systems. Both organisms exhibited a high prevalence of resistance to multiple antimicrobials. This has serious veterinary public health implications due to the negative impact on patient treatment and prognosis. Molecular studies are needed to identify genetic drivers of antimicrobial resistance among *A. baumannii* and *K. pneumoniae* organisms. In light of the high prevalence of AMR and MDR observed in this study, the need for strict infection prevention and control measures to prevent the transmission of these organisms in hospital settings cannot be overemphasised.

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Chapter 5

Occurrence and characterization of ESKAPE organisms on the hands of veterinary students before patient contact at a veterinary academic hospital, South Africa

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Abstract

Objective: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species termed 'ESKAPE' organisms are responsible for most hospital-acquired infections (HAIs). Although these organisms are known to spread via the hands of healthcare workers (HCWs), there is a paucity of information on the occurrence of these organisms in veterinary settings. The aim of this study was to determine the presence of ESKAPE organisms on the hands of students working in the intensive care unit (ICU) at a veterinary academic hospital.

Methods: A cross-sectional study was conducted among students working in an ICU at a veterinary academic hospital in South Africa. Students were sampled before the start of the ICU shift using a modified glove-juice method. Standard microbiological techniques and a series of polymerase chain reaction (PCR) assays were used to identify and characterize the bacteria. All the isolates were tested for resistance against a specific panel of antibiotics using the disk diffusion method. Proportions of bacterial species and their antimicrobial-susceptibility profiles were calculated.

Results: At screening, all the veterinary students (n=62) carried at least one of the ESKAPE organisms on their hands. *Escherichia coli* was the most isolated organism (76%, 47/62), followed by *E. faecium* (52%, 22/62), *P. aeruginosa* (48%, 30/62), *A. baumannii* (47%, 29/62), *K. pneumoniae* (27%, 17/62), and *S. aureus* (24%, 15/62). A reduced proportion of isolates were recovered from the samples, *E. coli* (26%, 12/47), *E. faecium* (27%, 6/22), *P. aeruginosa* (43%, 13/30), *A. baumannii* (21%,7/29), *K. pneumoniae* (41%, 7/17), and *S. aureus* (20%, 3/15). Most of the organisms showed a high proportion of resistance to at least one antibiotic. Multidrug resistance (MDR) was reported among (42%, 5/12) of *E. coli*, 40% (2/5) of *E. faecium*, 100% (13/13) of *P. aeruginosa*, and 33% (1/3) of *S. aureus* isolates.

Conclusion: Students working in the ICU carry several organisms from the ESKAPE group before contact with patients. Moreover, MDR was common among this group of organisms. The findings of the present study underscore the importance of infection prevention and control (IPC)

strategies to help reduce the likelihood of the spread of these organisms to personnel, owners, family members, and patients.

Keywords: ESKAPE pathogens, Veterinary, Intensive care unit, Antimicrobial resistance, Multidrug resistance, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species

5.1 Introduction

Effective hand hygiene has been shown to reduce the transmission of hospital-acquired infections (HAIs) in healthcare facilities (1–5). However, available evidence similar to studies in human medicine, indicates that hand hygiene compliance among healthcare workers (HCWs) in veterinary medicine remains low (6,7). This heightens the risk of transmission of infectious diseases and zoonotic organisms within the hospital setting (3,6,8). In addition to low hand hygiene compliance, patient-to-patient contact, and contact with contaminated surfaces have also been shown to increase the transmission of organisms associated with HAIs (4,9–11).

Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species referred to as ESKAPE are the leading cause of HAIs in the intensive care unit (ICU) in both human (12,13) and animal hospitals (14). Moreover, infections associated with these bacteria are less responsive to commonly used antibiotics resulting in limited treatment options and poor patient prognosis, especially in under-resourced developing countries (9–11,15). Additionally, some of these bacteria including enterococci, can survive in hospital environments for longer periods (approximately 5 days to 4 months) thus remaining a source of infection to susceptible individuals (10,11,16).

The intensive care unit of both the human and veterinary hospitals remains a high-risk area for infections associated with ESKAPE organisms due to the poor health status of the patients, the high antibiotic usage, the higher prevalence of invasive procedures, the use of indwelling devices, and the higher frequency of contact between patients and HCWs (11,17,18). This problem is compounded by the fact that animals and people can be carriers of these organisms, therefore, becoming sources of infections to susceptible individuals (19).

Given, hand hygiene compliance remains the most effective strategy to reduce the risk of transmission of organisms associated with HAIs in hospital settings (7,19–21). This study investigated the presence of ESKAPE bacteria on the hands of students working in the ICU at a veterinary academic hospital prior to contact with patients. The results shed light on the importance of hand hygiene compliance in the ICU setting.

5.2 Materials and methods

5.2.1 Study area

The study was conducted at a veterinary teaching hospital in South Africa. The faculty to which the hospital belongs has five departments: Veterinary tropical diseases, Paraclinical sciences, Companion animal clinical studies (CACS), Production animal clinical studies, and Anatomy and Physiology. This study focuses on the ICU servicing the Department of CACS. The Department has three sections: small animal surgery, small animal medicine, and outpatient. All patients from these sections requiring critical care are referred to the same ICU, excluding those with contagious infectious diseases like canine parvovirus, which are admitted to a separate isolation ward. In addition, the hospital serves as a referral for internal medicine and surgical cases for clients in and around Pretoria. The study was done during routine clinical rotations of veterinary students. The clinical rotation is divided into the day shift starting from 08h00 to 16:00 and night shifts starting from 20h00 to 08h00.

5.2.2 Study population

A cross-sectional study design was adopted in this study. Final-year students were sampled during their clinical rotation in the ICU between September 2022 and March 2023. The students were randomly selected on different days based on the shift as they entered the ICU at the start of the shift. Each student was sampled once.

5.2.3 Sample collection

The study used the glove-juice technique which is well documented in human medicine studies (1,14,21). This method is more sensitive compared to the imprint method as it allows for the quantification of the entire bacterial load on the hands of the HCWs (22,23). To sample for the presence of ESKAPE organisms, the dominant hand of each participant was inserted into a sterile latex-free glove containing 20ml buffered phosphate water (PBW) and massaged for one minute as described by (25) Matuka et al (24). After massaging, the fluid was aseptically retrieved and pipetted

into sterile 15ml tubes then transported on ice within an hour to the veterinary public health (VPH) laboratory of the faculty of veterinary science for further processing.

5.2.4 Screening

Samples brought to the laboratory in PBW were incubated in a shaker at 200 RPM for 16-24 hours at 37°C. After enrichment, 100µl aliquot of the overnight broth was spread on horse blood agar and incubated aerobically at 37°C for 16-24 hours.

5.2.5 Identification of ESKAPE bacteria

5.2.5.1 DNA Extraction

All blood agar plates with growth: the bacterial colony was harvested using a sterile loop in preparation for extraction of genomic Deoxyribose nucleic Acid (DNA) using the boiling method as previously described (25). A loopful of the culture sweep was suspended in 1000µL of sterile FA buffer (BactoTM FA Buffer, Becton and Dickson &Company) in a 1.5mL Eppendorf tube, vortexed and centrifuged at 12,000rpm for 5 minutes. The supernatant was discarded, and the bacterial pellet was re-suspended in 1000µL of sterile FA buffer and centrifuged. This process was repeated twice. After the last centrifugation cycle, the supernatant was discarded completely. The pellet was re-suspended in 500µL of sterile distilled water, boiled for 20 minutes on a heating block, cooled on ice for 10 minutes, and then stored at -20^{0}C for further processing.

5.2.5.2 Polymerase Chain Reaction

The extracted genomic DNA was used as a template to determine the presence of each of the ESKAPE organisms using polymerase chain reaction (PCR). Primers and PCR cycling conditions as previously described were used to identify the different bacteria **(Table 5.1)**. Briefly, for each PCR reaction of 25µL, the following components were added: 2.5µL of 10X Thermopol reaction buffer, 2.0µl of 2.5mM dNTPs (deoxynucleotide triphosphates), 0.25µl of 100mM MgCl2, 1.6µl of each primer (0.64µM final concentration), 1U of Thermus aquaticus polymerase (Taq) DNA Polymerase (New England BioLabs® Inc.) and 5µl of DNA lysate template. Positive controls included DNA from the ATCC strains *E. coli* (25922), *S. aureus* (25923), *K. pneumonia* (700603), *E. faecalis*

(29212), and *P. aeruginosa* (27853). Sterile nuclease-free water was used as a negative control. All PCR reagents were supplied by New England BioLabs (NEB, USA), except for the primers, which were sourced from Inqaba Biotec (South Africa) and Integrated DNA Technologies (IDT) (San Diego, USA).

A Veriti™ (Applied Biosystems®, USA) or a C1000 TouchTM (Bio-Rad, USA) thermal cycler was used for all PCR reactions. Thereafter, the PCR products were electrophoresed on 2% (w/v) agarose gels in TAE (Tris–acetate–ethylenediamine tetra acetic acid) buffer, stained with ethidium bromide (0.05mg/µl) for 15 minutes, and visualized under ultraviolet (UV) light with a Gel Doc system (Bio-Rad, USA).

Table 5. 1: Nucleotide sequences used as primers in the PCR reaction to identify ESKAPE organisms.

organism	Primer Sequences	Amplicon size [a](bp)	Reference
Enterococcus faecium	[b]F:GAAAAAACAATAGAAGAATTAT [c]R:TGCTTTTTTGAATTCTTCTTTA	215	(26)
Staphylococcus aureus	[b]F:AATCTTTGTCGGTACACGATATTCTTCACG [c]R:CGTAATGAGATTTCAGTAGATAATACAACA	108	(27)
Klebsiella pneumoniae	[b]F:GGATATCTGACCAGTCGG [c]R:GGGTTTTGCGTAATGATCTG	176	(28)
Acinetobacter baumannii	[b]F: CACGCCGTA-AGAGTGCATTA [c]R: AACGGAGCTTGTCAGGGTT	490	(29)
Pseudomonas aeruginosa	[b]F: AATACCTTGCTGTTTTGACGTTAC [c]R:TCAGTGTCAGTATCAGTCCAGGTG	295	(30)
Escherichia coli	[b]F:GATGAAATGGCGTTGGCGCAAG [c]R:GGCGGAAGTCCCAGACGATATCC	373	(31)

[a]Base pairs, [b]Forward primer, [c]Reverse primer

5.2.5.3 Single Colony Streaking

Plates that tested positive in the initial screening were streaked onto differential media to differentiate each bacterium to obtain single colonies. *Staphylococcus aureus* and *A. baumannii* were streaked on blood agar, *P. aeruginosa* on Cetramide agar, and *E. faecium, E. coli* and *K. pneumoniae* were streaked on McConkey agar. The plates were then incubated at 37^0C for 16-24 hours. Five single colonies of each isolate were selected from each plate and multiplied separately

on Luria Bertani (LB) agar (DifcoTM Becton and Dickson & Company) for purification. The plates were then incubated at 37^0C for 16-24 hours. Genomic DNA was extracted, and PCR was performed on the colonies using primers as described above to identify them.

5.2.6 Antimicrobial sensitivity

All the identified isolates were tested against a panel of antibiotics using the disk diffusion method to determine their susceptibility profile following the Clinical and Laboratory Standards Institute (CLSI) guidelines **(Table 5.2)** (32).

Antimicrobial resistance testing was performed on Mueller Hinton agar (MHA) (Oxoid, UK) as described by the CLSI (32). Bacterial suspensions of individual pure colonies of 0.5 McFarland were prepared in 0.85% physiological saline. A sterile cotton swab was used to inoculate MHA plates to achieve confluent growth. Antimicrobial discs were placed on the inoculated plates using an Oxoid disk dispenser and incubated aerobically at 37^0C for 16-24 hours. Each isolate was tested against different panels of antibiotics using disks obtained from Oxoid Company as outlined in **Table 5.2.** *Escherichia coli* (25922), *S. aureus* (25923), *K. pneumonia* (700603), *E. faecalis* (29212), and *P. aeruginosa* (27853) were used as reference strains. The results of the antibiogram were classified as susceptible, resistant, or intermediate according to CLSI criteria (32). However, the intermediate readings were re-classified as resistant for data analysis.

Table 5. 2: Panel of antibiotics tested against the ESKAPE organisms isolated from the hands of healthcare workers in the intensive care unit.

Antibiotics	*Enterococcus faecium*	*Staphylococcus aureus*	*Klebsiella Pneumoniae*	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*	*Escherichia coli*
Ampicillin (10µg)	✓		✓		✓	✓
Penicillin-G (10µg)	✓	✓			✓	
Cefotaxime (30µg)			✓	✓	✓	✓
Tobramycin (10µg)			✓	✓	✓	✓
Ciprofloxacin (5µg)	✓	✓	✓	✓	✓	
Ceftazidime (30µg)			✓	✓	✓	✓
Ampicillin-sulbactam (10/10µg)			✓	✓	✓	✓
Gentamicin (10µg)	✓	✓	✓	✓	✓	✓
Imipenem (10µg)	✓	✓	✓	✓	✓	✓
Trimethoprim-sulfamethoxazole (25µg)	✓	✓			✓	
Amikacin (30µg)					✓	
Oxytetracycline (30µg)		✓	✓	✓		
Erythromycin (15µg)	✓	✓				
Chloramphenicol (30µg)	✓	✓				✓
Linezolid (30µg)		✓				
Oxacillin (1µg)		✓				
Tetracycline (30µg)	✓	✓				✓
Total antibiotics	**9**	**11**	**9**	**8**	**11**	**9**

5.3 Results

5.3.1 Isolated bacteria

Sixty-two (n=62) students gave consent to be sampled, and all the students who participated in the study, carried at least one of the ESKAPE organisms on their hands. *Escherichia coli* (76%) was the most identified bacteria and *S. aureus* (24%) was the least identified during the screening. A reduced proportion of isolates were recovered from single colony streaking (**Table 5.3)**.

5.3.2 Antimicrobial susceptibility profile

All the isolated ESKAPE organisms exhibited a high proportion of resistance to at least one antibiotic. Among the *E. coli* isolates, resistance was high to ampicillin, cefotaxime, and tobramycin. While two of the three *S. aureus* isolates exhibited resistance to penicillin G. Most *K. pneumoniae* isolates were resistant to ampicillin and none were resistant to ceftazidime, gentamycin, and imipenem. *Acinetobacter baumannii* isolates exhibited resistance to ampicillin-sulbactam and one isolate showed resistance to imipenem. All *P. aeruginosa* isolates showed resistance to ampicillin, penicillin-G, and ampicillin-sulbactam, three of the isolates were resistant to imipenem, and two to tobramycin. Three *Enterococcus faecium* isolates were resistant to penicillin-G and two to ciprofloxacin, erythromycin, and ampicillin **(Table 5.4)**.

5.3.3 Multidrug-resistant Organisms

Only *E. coli, P. aeruginosa, E. faecium,* and *S. aureus* had isolates that were resistant to three or more antibiotics and thus considered MDR **(Table 5.3)**

Table 5. 3: The proportions of bacteria isolated from the hands of students before contact with patients in the intensive care unit at a veterinary academic hospital; South Africa

	Isolates		Resistant Isolates	
Bacterial organism	**Screening % (n/N)**	**Recovered % (n/N)**	**AMR[b] % (n/N)**	**MDR[c] % (n/N)**
Enterococcus faecium	35 (22/62)	23 (5/22)	80 (4/5)	40 (2/5)
Staphylococcus aureus	24 (15/62)	20 (3/15)	67 (2/3)	33 (1/3)
Klebsiella pneumoniae	27 (17/62)	41 (7/17)	100 (7/7)	0 (0/7)
Acinetobacter baumannii	47 (29/62)	24 (7/29)	57 (4/7)	0 (0/7)
Pseudomonas aeruginosa	48(30/62)	43 (13/30)	100 (13/13)	100 (13/13)
Escherichia coli	76 (47/62)	26 (12/47)	100 (12/12)	42 (5/12)

[b]Antimicrobial resistance, [c]Multidrug resistance

Table 5. 4: Antimicrobial resistance profile of ESKAPE organisms isolated from hand samples of students working at a veterinary academic hospital, in South Africa.

Antibiotics	*Enterococcus faecium* **% (n/N)**	*Staphylococcus aureus* **% (n/N)**	*Klebsiella Pneumoniae* **% (n/N)**	*Acinetobacter baumannii* **% (n/N)**	*Pseudomonas aeruginosa* **% (n/N)**	*Escherichia coli* **% (n/N)**
Ampicillin	40 (2/5)		86 (6/7)		100 (13/13)	89 (8/9)
Penicillin-G	60 (3/5)	67 (2/3)			100 (13/13)	
Cefotaxime			14 (1/7)	25 (1/4)	69 (9/13)	67 (6/9)
Tobramycin			14 (1/7)	0 (0/4)	15 (2/13)	56 (5/9)
Ciprofloxacin	40 (2/5)	0 (0/3)	14 (1/7)	0 (0/4)	0 (0/13)	
Ceftazidime			0 (0/7)	25 (1/4)	0 (0/13)	44 (4/9)
Ampicillin-sulbactam			14 (1/7)	50 (2/4)	100 (13/13)	33 (3/9)
Gentamicin	0 (0/5)	0 (0/3)	0 (0/7)	25 (1/4)	69 (9/13)	22 (2/9)
Imipenem	0 (0/5)	0 (0/3)	0 (0/7)	25 (1/4)	23 (3/13)	
Trimethoprim-sulfamethoxazole	0 (0/5)	0 (0/3)			69 (9/13)	
Amikacin					0 (0/13)	
Oxytetracycline		33 (1/3)	0 (0/7)	0 (0/4)		
Erythromycin	40 (2/5)	33 (1/3)				
Chloramphenicol	0 (0/5)	0 (0/3)				11 (1/9)
Linezolid		0 (0/3)				
Oxacillin		0 (0/3)				
Tetracycline .	40 (2/5)	33 (1/3)				44 (4/9)

5.4 Discussion

This is the first study in South Africa to investigate the occurrence of ESKAPE organisms from the hands of HCWs in a veterinary hospital and their antimicrobial susceptibility profiles. During screening, at least one of the ESKAPE organisms was isolated from the hands of students before entering the ICU. The presence of these bacteria is concerning as they are known to cause opportunistic infections and are responsible for many HAIs (10,11,33–38). Moreover, these bacteria have zoonotic potential and can be transmitted between humans and animals, posing a health threat to susceptible individuals (16,38). The high prevalence of antimicrobial resistance observed among the isolates is also a matter of public health concern. The danger caused by these bacteria to public health is exacerbated by the fact that they can adapt and survive in hospital environments (12,38).

5.4.1 Escherichia coli, Klebsiella pneumoniae, and Enterococcus faecium

In the current study, *E. coli* was isolated from 76% of students working in the ICU. This is consistent with what other studies have reported the *E. coli* from the fingertips of HCWs in a human hospital (24) and the hands of HCWs in a veterinary hospital (39). *Klebsiella pneumoniae* and *E. faecium* were also isolated in this study. A study done in a small animal hospital in Korea (10) also reported the occurrence of these bacteria on the hands of HCWs. Of interest is that *K. pneumoniae* and *E. faecium* have been isolated from equipment and the hospital environment in other studies (16,40). The presence of these pathogens on environmental surfaces has been associated with faecal contamination (10,11,39). Therefore, it is important to implement measures that reduce the risk of faecal contamination, such as regularly cleaning and disinfecting surfaces within the veterinary hospital.

5.4.2 Staphylococcus aureus, Acinetobacter baumannii, and Pseudomonas aeruginosa

Staphylococcus aureus and *A. baumannii* are commensals on the skin of humans and animals as well as human nasal cavities (12,16,24,41). They are among the most prevalent opportunistic organisms in both human and veterinary hospitals (12). Humans remain important reservoirs for the transmission of these organisms (42). Similar findings have also been observed by other studies that investigated these bacteria from the hands of HCWs (11,24,43,44).

Concerning *P. aeruginosa,* to our knowledge, this is the first study in 20 years to report the occurrence of *P. aeruginosa* in the hands of HCWs in veterinary medicines, previous reports were on veterinary clinical cases and the environmental samples (45,46). The use of alcohol-based hand rubs and gels remains the most effective method of reducing the transmission of *S. aureus, A. baumannii,* and *P. aeruginosa* in hospital settings (24,42,47).

5.4.3 Antimicrobial resistance

Resistance against β-lactams was observed among *Enterococcus faecium, S. aureus, K. pneumoniae, P. aeruginosa, and E. coli* isolates which is consistent with what other studies have reported (48,49). However, the presence of resistance to imipenem in one *A. baumannii* and three *P. aeruginosa* isolates was concerning, given that imipenem is considered a high priority critically important antibiotic by the World Health Organization (WHO) (34,46,48,50).

Multidrug resistance was observed among *E. coli, P. aeruginosa, E. Faecium,* and *S. aureus* isolates. This was expected in light of reports by various studies that have demonstrated that ESKAPE organisms tend to exhibit high levels of resistance against commonly used antibiotics including the last resort antibiotics (38,48,50). Ng et al (51) also isolated MDR *A. baumannii* and MDR *E. coli* from doorknobs, labcoats, stethoscopes, and weighing scales. The observed MDR among these organisms implies the heightened likelihood of treatment failure among patients if they contracted HAIs (11,49,52).

5.5 Conclusion

Students in this study carried on their hands bacteria associated with HAIs and zoonotic diseases. These bacteria exhibited a high prevalence of resistance to the β-lactams antibiotics and two of them were resistant to imipenem. Therefore, veterinary hospitals should prioritize pathogen surveillance to control the spread of MDR organisms. Since these organisms are opportunistic and likely to survive in harsh environments, adherence to hand hygiene and other IPC practices at the veterinary academic hospital is recommended.

5.6 Declarations

5.6.1 Ethics approval and consent to participate

The Faculty of Veterinary Science Research Ethics Committee, Faculty of Humanities Research Ethics Committee (Project number: REC009-21), and Faculty of Health Sciences Research Ethics Committee (Reference No:187/2022) approved this study. Students were informed of the study during their clinical orientation week and gave consent before participating. All the data was kept anonymous for confidentiality.

5.6.2 Consent for publication

Not applicable

5.6.3 Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

5.6.4 Competing interests

The authors declare that they have no competing interests.

5.6.5 Funding

There was no external funding received for this study.

5.6.6 Authors' Contribution

DCS, JWO, MK, and DNQ have made a substantial contribution to the conception and design of the study. DCS was involved in the acquisition, initial analysis, and interpretation of data, and the drafting of the article. JWO, MM, MK, and DNQ were involved in the extensive review of the manuscript. All the authors read and approved the final version of the manuscript.

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Chapter 6

Knowledge of veterinary students on the transmission of hospital-acquired infections and zoonotic diseases at the veterinary academic hospital

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My contribution to the paper includes study design, data analysis, interpretation of results, writing of manuscript as well as extensive editing of the manuscript

Abstract

Background: Compliance with hand hygiene measures remains low among healthcare workers (HCWs) in veterinary medicine. The knowledge about infection prevention and control (IPC) is among the factors that drive the low level of hand hygiene compliance. This study assesses the knowledge of IPC and the transmission of hospital acquired infections (HAIs) among veterinary students entering the final year of clinical training.

Methods: The questionnaire survey was conducted among 147 final-year veterinary students at the Faculty of Veterinary Science addressing knowledge of the transmission of organisms associated with HAIs, zoonotic diseases, and IPC. The format of responses included yes or no and 5-point Likert scale answers. The Likert scale responses ranged from “Strongly disagree” to “Strongly agree. Proportions of categoric variables and 95% confidence intervals were calculated and presented in tables.

Results: Of the 147 students interviewed, most were female (69.4%) followed by male (29.2%). Most (59.2%) of the respondents indicated they had not heard about IPC programs, and it was emphasized before the start of the clinical training (92,2%, 130/141) and the clinical rotation (91,9%, 103/112). Thirty-nine percent (54/137) of respondents indicated the topic of transmission of HAIs was lightly emphasized pre-clinical and 43% (47/110) indicated that the topic was emphasized in multiple courses during clinical training. Students indicated that they were adequately trained on hand hygiene compliance, cleaning and disinfection, and the use of personal protective equipment (PPE). However, half indicated they were not adequately trained in IPC regarding patient management and disease surveillance. However, students knew that jewellery, stethoscopes, ward telephones, and leashes are possible sources of pathogens associated with HAIs.

Conclusion: Veterinary students have a poor understanding of IPC practices in this study. The students also reported that training on the topic is not given enough attention during lectures compared to during clinical training. Therefore, teaching and training at the undergraduate level must continue to ensure that students are adequately capacitated on IPC.

6.1 Introduction

The transmission of most organisms associated with hospital-acquired infections (HAIs) is mostly through the contaminated hands of healthcare workers (1,2). Studies in both human and veterinary medicine have demonstrated that effective hand hygiene compliance such as hand washing using water and soap and disinfecting hands with alcohol-based hand sanitizers (ABHS) reduce the transmission of bacteria associated with HAIs and zoonotic diseases. However, compliance among healthcare workers remains low in both humans and animals (1–7). In South Africa, Sebola et al (8) also reported poor infection prevention and control (IPC) practices such as the use of personal phones and the wearing of wristwatches among hospital personnel when attending to patients.

Studies attribute the low level of compliance to high workload, lack of resources, underlying clinical conditions including skin irritation, forgetfulness, and lack of knowledge (3,4,7,9). The absence of written IPC protocols in hospitals and the lack of awareness among medical staff are also considered one of the drivers of low levels of hand hygiene compliance (10). Katz-Hulana (11) suggests that implementing standardized IPC strategies across clinical rotations will likely improve the overall level of hand hygiene compliance. Furthermore, emphasizing IPC measures at an undergraduate level may assist in improving the knowledge, attitude, as well as practice of students on hand hygiene (8,10,12).

Knowledge, attitude, and practice (KAP) surveys are important in providing useful baseline data to inform awareness strategies on IPC and guide interventions for the reduction of HAIs and zoonotic pathogens in veterinary facilities (13). Therefore, this study aims to investigate the knowledge of veterinary students regarding the transmission of HAIs in the intensive care unit (ICU). Information collected will be used to identify knowledge gaps and behavioral patterns to guide the development of intervention programs including curriculum change.

6.2 Materials and methods

6.2.1 Study area and study population

This study was conducted among the final year veterinary students at the University of Pretoria, Faculty of Veterinary Science in South Africa. The faculty is the only one in South Africa

doing training in veterinary medicine. Veterinary science is a 6-year program, consisting of 4 years and 6 months of didactic teaching and 18 months of clinical training. This study targets final-year veterinary students (147) in their clinical training program.

6.2.1.1 Questionnaire design

The questionnaire was designed in Epi Info™ and consisted of closed questions in the form of checklists and selection types. It included questions that assessed the students' knowledge and of the transmission of organisms associated with HAIs, zoonotic diseases, and IPC in veterinary settings. The formats of responses were yes or no and 5-point Likert scale answers. The range of the Likert scale responses were "Strongly disagree = 1, Disagree = 2, Neither agree nor disagree = 3, Agree = 4, and Strongly agree = 5". The questionnaire was adapted from the one by the World Health Organization (14). Pre-testing of the questionnaire was done among employees, students, and clinicians at the Faculty of Veterinary Sciences. Where necessary the questionnaire was modified to improve its quality and accuracy.

6.2.1.2 Data Collection

The questionnaire survey was conducted in July 2022. An information session was held by the principal investigator (DC) with all the students explaining the objective of the study. All students who attended the session were given hard copies of the questionnaires. The survey was estimated to take approximately 10 to15 minutes to complete.

6.2.1.3 Data management and Data analyses

The data from hard copies was captured using Epi Info™(15) and stored as a MicroSoft access file type. Before the analysis, the data was assessed for any inconsistencies. Proportions of categoric variables and 95% confidence intervals were calculated and tabulated using IBM SPSS Statistics (Version 29.0.0.0(241)). The analysis and interpretation of the 5-point Likert scale were done as shown in **Table 6.1**.

Table 6. 1: The analysis and interpretation of the 5-point Likert scale based on the weighted average of students working in the ICU at a veterinary academic hospital.

Weighted average	Results	Interpretation
1-1.8	Strongly disagree	Very low perception
1.81-2.6	Disagree	Low perception
2.61-3.4	Neutral	Neutral
3.41-4.2	Agree	Perception
4.21-5.0	Strongly agree	High perception

6.2.2 Ethics and confidentiality

This study was approved by the Faculty of Veterinary Sciences Research Ethics Committee (REC009-21) and the University of Pretoria Survey Coordinating Committee. Consent forms were given to students before the beginning of the study and students were free to decline participation in the study. Participants were requested not to include their names or any form for anonymity.

6.3 Results

Of the 147 students interviewed most were female (69.4%, 102/147) followed by male (29.2%, 43/147). Two (1.4%, 2/147) students did not indicate their sex.

6.3.1 Knowledge of respondents on infection prevention control program

Most (59.2%) of the respondents indicated they had not heard about IPC programs. Of those who heard about IPC practices, 81% indicated they heard during lectures. Almost 82% of respondents indicated that the topic of infection prevention and control was emphasized before start of the clinical training. A similar proportion (80%) of respondents indicated that IPC is emphasized during the clinical rotation. Based on the results, the respondents were adequately trained on hand hygiene compliance, cleaning and disinfection, and the use of PPE. Almost half of the respondents indicated they were not adequately trained in IPC as it relates to patient management and disease surveillance.

Of those who indicated that the topic of transmission of HAIs was covered during the pre-clinical training, almost forty percent (39.4%) indicated that the topic was lightly emphasized. While 42.7% of the respondents indicated that the topic was emphasized in multiple courses during clinical rotations (**Table 6. 2).**

Table 6. 2: Questions relating to the knowledge of students on infection prevention and control as well as hospital-acquired infections.

Variable	Frequency	Percentages	[d]CI
Have you heard of the infection prevention and control program (N=147)			
Yes	60	40.8	33.2-48.9
No	87	59.2	51.1-66.8
Where did you hear (n=54)			
Class	49	90.7	80.09-95.98
Word of mouth	3	5.6	1.91-15.11
Clinical training	1	1.9	0.33-9.77
Online	1	1.9	0.33-9.77
Is Infection prevention and control emphasized pre-clinics (n=141)			
Topic not covered	11	7.8	4.41-13.43
Lightly emphasized	46	32.6	25.44-40.73
Only covered in one course	41	29.1	22.22-37.05
Only covered in multiple courses	43	30.5	23.5-38.53
Is Infection prevention and control emphasized during clinics (n=112)			
Topic not covered	9	8.0	4.28-14.57
Lightly emphasized	36	32.1	24.21-41.26
Only covered in one course	26	23.2	16.36-31.84
Only covered in multiple courses	41	36.6	28.27-45.83
Which of the following infection prevention and control practices have you professionally trained[e] (n=147)			
Hand hygiene	121	82.3	75.35-87.63
Cleaning and disinfection	109	74.1	66.29-81.01
Use of personal protective equipment	109	74.1	66.29-81.01
Patient management	82	55.8	47.71-63.56
Disease Surveillance	76	51.7	43.68-59.63
None	9	6.1	3.25-11.23
Is the transmission of hospital-acquired infections emphasized in the pre-clinical syllabus (n=137)			
Topic not covered	10	7.3	4.01-12.91
Lightly emphasized	54	39.4	31.63-47.78
Only covered in one course	35	25.5	18.98-33.45
Emphasized in multiple courses	38	27.7	20.93-35.76
Is the transmission of hospital-acquired infections emphasized in your clinical training year (n=110)			
Topic not covered	12	10.9	6.35-18.1
Lightly emphasized.	31	28.2	20.62-37.21
Only covered in one course	20	18.2	12.09-26.42
Emphasized in multiple courses	47	42.7	33.88-52.06

[d]Confidence interval
[e]Trained at an accredited or registered training institution

Most of the respondents highly perceived the use of personal cellphones, thermometers, and contaminated hands of HCWs as routes for the transmission of organisms associated with HAIs. Respondents also perceived the wearing of jewellery, use of stethoscope, use of ward telephone, and use of leashes as possible routes for the transmission of pathogens associated with HAIs **(Table 6.3)**.

Table 6. 3: The perception of students on whether equipment could lead to possible transmission of hospital-acquired infection**.**

Items (n=143)	SD[a]		D[b]		NA[c]		A[d]		SA[e]		Mean	Decision
	n	%	n	%	n	%	n	%	n	%		
Cellphone	1	0.7	2	1.4	6	4.2	55	38.5	79	55.2	4.46	High perception
Jewellery	3	2.1	10	7.0	15	10.5	70	49.0	45	31.5	4.01	Perception
Stethoscope	0	0	5	3.5	19	13.3	62	43.4	57	39.9	4.20	Perception
Thermometer	0	0	2	1.4	6	4.2	37	25.9	98	68.5	4.62	High perception
Ward telephone	1	0.7	11	7.7	21	14.7	63	44.1	47	32.9	4.01	Perception
Use of leashes	1	0.7	7	4.9	18	12.6	67	46.9	50	35.0	4.10	Perception
Contaminated hands	0	0	0	0	1	0.7	27	19.0	114	80.3	4.80	High perception

[a]Strongly disagree, [b]Disagree, [c]Neither agree nor disagree, [d]Agree, [e]Strongly agree

6.4 Discussions

This study aimed to investigate the knowledge of veterinary students on IPC practices, and transmission of organisms associated with HAIs. As the first study in South Africa to investigate this area, the data collected will be used to guide intervention strategies for IPC in the veterinary academic hospital.

6.4.1 Knowledge of respondents on infection prevention control program and hospital acquired infections

The students in this study had a low overall knowledge of IPC programs. In contrast, studies have reported good knowledge of IPC among HCWs in human hospitals (16–18). The low knowledge among the students in this study is puzzling as the same students indicated that IPC and topics on HAIs were covered during clinical and pre-clinical training although in varying degrees. The lengthy duration of the veterinary program could also be contributing to students' recall bias, leading to a higher recall of emphasis during clinics as the information is recent (19). Nonetheless, the results seem to suggest that student do not have a good understanding of infection prevention and control measures and their role in the prevention of HAIs and zoonotic diseases. Since most students indicated that their knowledge of IPC was acquired during lectures, emphasis on IPC must be done both in undergraduate lectures and during clinical training (13,20).

It is noteworthy that a few respondents indicated they received training in disease surveillance and patient management when compared to other IPC strategies. This is not surprising, as there is insufficient reporting on the knowledge of HCWs on disease surveillance and patient

management in relation to IPC (21–23). The authors hypothesise that this could be due to the integrated nature of teaching and training on patient management and disease surveillance rather than a standalone module. Moreover, the current undergraduate syllabus contains an epidemiology module that includes disease surveillance. Therefore, an undergraduate curriculum review is needed to identify potential gaps and make recommendations for changes if needed.

Students agreed that cellphones, thermometers, jewellery, stethoscopes, ward telephone, leashes and contaminated hands could be potential sources for the transmission of organisms associated with HAIs. Although the expectation is that this will translate to behavioral change. Studies have shown that a high perception of IPC measures does not correspond with compliance (8,13,16). Duerink et al (24) in Indonesian. reported better compliance among HCWs with more knowledge compared to those with less knowledge of IPC. Therefore, knowledge of IPC measures alone is not enough to improve compliance, other factors must also be considered (25).

6.5 Conclusion

Student do not have a good understanding of IPC measures and their role in the prevention of HAIs and zoonotic diseases in this study. It is possible that the integrated nature of teaching and training could be contributing to a lack of understanding, therefore, a review of the undergraduate curriculum could be helpful in bridging the gap. Of noteworthy is that students are aware that the different equipment used during patient care could be potential sources for organisms associated with HAIs.

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Chapter 7 Summary of Discussions and Recommendations

7.1 Discussions and conclusions

This chapter reviews the objectives of the study and summarizes the key findings. It also provides conclusions and recommendations for future research. The study aimed to assess the pre-intervention IPC practices required to reduce the transmission of organisms associated with hospital-acquired infections (HAIs) and zoonotic diseases at a veterinary hospital. The pre-intervention assessments included the level of knowledge of veterinary students on HAIs, identification of organisms associated with HAIs and zoonotic diseases from the hands of healthcare workers (HCWs). The information generated from this study will contribute to a better understanding of the epidemiology of zoonotic and HAI organisms in veterinary medicine. The objectives of this study were: (1) To describe organisms associated with HAIs and zoonotic infections and their antimicrobial-resistant patterns in veterinary hospitals; (2) To describe the antimicrobial resistance patterns of *Klebsiella pneumoniae* and *Acinetobacter baumannii* from clinical samples of dogs presented to a veterinary academic hospital in South Africa between 2007 and 2013; (3) To Investigate the Knowledge of students on the transmission of HAIs in the intensive care unit (ICU); (4) To investigate the occurrence of organisms associated with HAIs on the hands of students working in the ICU; and (5) describe their antimicrobial resistance patterns.

Studies have suggested that improving the surveillance system is critical in the prevention of HAIs and in reducing the emergence of antimicrobial-resistant pathogens. Bacterial organisms associated with HAIs and zoonotic diseases were reported from clinical cases, environmental surfaces, and commonly used equipment in veterinary settings. The hospital environment with human contact was the most reported source of bacteria associated with HAIs. These results suggest that humans play a crucial role in transmitting HAIs in veterinary hospitals. Among the bacteria reported, Methicillin-resistant *Staphylococcus aureus* was the most reported HAI bacteria in veterinary facilities. Other bacteria identified *include* Methicillin-resistant *Staphylococcus pseudintermedius, Escherichia coli, Clostridium difficile, Acinetobacter baumannii, Salmonella* species, and *Enterococcus* spp. Some of these isolates reported in veterinary settings share similar clonal lineage to those reported in humans. Some bacteria exhibited a high prevalence of

antimicrobial resistance and contain genes known to be associated with antibiotic resistance. From these results, the author recommends strict and continuous infection prevention and control (IPC) practices in veterinary medicine. In addition, veterinary hospitals must implement continuous surveillance of organisms associated with HAIs and their antimicrobial resistance patterns.

In South Africa, studies of ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species) bacteria have been well-documented in human medicine. However, studies investigating antimicrobial drug resistance among the ESKAPE group in veterinary medicine are limited. In the current study, multidrug resistance (MDR) *A. baumannii* and *K. pneumoniae* were identified from various tissues such as bone, urine, skin, blood, ear, nasal, bronchoalveolar lavage, and oral cavity isolated from dogs admitted in a veterinary hospital in South Africa. This suggests that these pathogens are associated with various clinical infections in dogs and can infect different body systems. In addition, these bacteria exhibited a high level of resistance to commonly used antibiotics for treatment in small animal practices. *Acinetobacter baumannii* showed resistance towards antibiotics from classes of β-lactams, cephalosporins, and trimethoprim-sulphamethoxazole. However, a low prevalence of resistance was observed against antibiotics from classes such as carbapenems, colistin, and fluoroquinolones. These results as well as combination antimicrobials suggest the possible choice of treatment for MDR *A. baumannii*. *Klebsiella pneumoniae* showed low resistance towards imipenem, amikacin, gentamycin, and trimethoprim-sulphamethoxazole, which can still be investigated for treatment options.

Furthermore, this study isolated ESKAPE organisms from the hands of students working in the ICU before patient contact. *Escherichia coli, Klebsiella pneumoniae,* and *Enterococcus faecium* were among the bacteria identified. Similarly, *Staphylococcus aureus, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* were also isolated in this study. This suggests that human-environment interaction could have led to hand contamination. Therefore, the isolation of these bacteria indicates that effective hand hygiene compliance in the veterinary hospital should be emphasized. In addition, veterinary environmental surfaces and commonly touched equipment are to be thoroughly cleaned and disinfected after every use to minimize the bacterial load, especially on surfaces with constant human contact.

Most of the isolated ESKAPE organisms in this study were resistant to the β-lactams and *A. baumannii* and *P. aeruginosa* showed resistance towards imipenem. Furthermore, MDR was observed in *E. coli, P. aeruginosa, E. Faecium,* and *S. aureus*. The presence of MDR bacteria is likely to impact treatment options and patient outcomes in zoonotic and HAI cases, therefore, antimicrobial stewardship must be prioritized in veterinary hospitals.

The knowledge of IPC practices seems to be low among the students while knowledge of the potential instrument that can lead to the transmission of organisms associated with HAIs in the ICU was adequate. However, students lacked an understanding of individual IPC measures and suggested the pre-clinical program was not adequate. Therefore, recommends a review of the undergraduate curriculum as it could be helpful in bridging the gap.

The results of this study provide baseline data for understanding the prevalence and antimicrobial sensitivity profile of bacteria associated with HAIs in veterinary medicine. In addition, the information generated will contribute to the development and implementation of the South African national AMR framework.

7.1.1 Limitations of the study

The study is not without limitations; for example,

1. This study was limited to one veterinary hospital and did not include other veterinary medical facilities, thus the results cannot be generalized to the entire South African veterinary medicine sector.
2. This study of knowledge focused only on veterinary students and not all veterinary HCWs were interviewed or sampled.
3. The data for the knowledge survey were self-reported which could be subject to the respondent's memory and biases.

7.2 Recommendations

In line with the multimodal strategies to promote effective IPC practices the following strategies are proposed:

1. **Hand hygiene training** should be provided to both veterinary students and other HCWs. The training can be conducted through presentations, discussions, and demonstrations. The

key concepts of the training should include, but not be limited to, epidemiology, the incidence and burden of MDR bacteria (ESKAPE) within veterinary medicine, the national strategic plan on antimicrobial resistance, and one health concept.

2. **Educational and reminder posters** should be created and placed in specific areas of the veterinary hospital. These areas include entry doors, locations where hand hygiene is crucial (such as near sinks), and treatment rooms. The posters should provide information about the five hand hygiene moments and emphasize the significance of IPC practices in reducing the spread of MDR bacteria.
3. **Different educational platforms** should be established and utilized to spread the knowledge of IPC practices. An example could be social media platforms.
4. **Infection prevention and control champions** should be identified and selected based on the need and area of expertise.
5. **Regular feedback on IPC inspections and audits** must be done. The feedback session should be conducted by the IPC champion and can be done during group meetings.

Chapter 8 Annexures

Table 8. 1: List of documents in the annexure section with chapters associated.

Document	Chapter associated
Publication 1	Chapter 3
Risk of Bias	Chapter 3
Publication 2	Chapter 4
Questionnaire Survey	Chapter 6
REC Renewal approval	All Chapters
Ethical approval amendment	All Chapters
Survey committee approval	Chapter 6
Section 20 approval	All Chapters
Humanities ethics approval	All Chapters
Health Science ethics approval	Chapter 5

UNIVERSITEIT VAN PRETORIA
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Faculty of Veterinary Science

Research Ethics Committee 4 August 2022

AMENDMENT LETTER OF APPROVAL

Ethics Reference No	**REC009-21 Line 1**
Protocol Title	**Multimodal approaches to reducing transmission of hospital-acquired infections (HAI) in the intensive care unit (ICU) at a veterinary academic hospital**
Principal Investigator	**Ms DC Sebola**
Supervisors	**Prof DN Qekwana**

Dear Ms DC Sebola,

We are pleased to inform you that the **Amendment** conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC009-21) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

CONDITIONALLY APPROVED NOTE: UP Survey committee approval letter still to be uploaded

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

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Faculty of Veterinary Science

Research Ethics Committee 12 April 2023

ANNUAL RENEWAL LETTER OF APPROVAL

Ethics Reference No	**REC009-21 Line 2**
Protocol Title	**Multimodal approaches to reducing transmission of hospital-acquired infections (HAI) in the intensive care unit (ICU) at a veterinary academic hospital**
Principal Investigator	**Ms DC Sebola**
Supervisors	**Prof DN Qekwana**

Dear Ms DC Sebola,

We are pleased to inform you that the **Annual Renewal** conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC009-21) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

PLEASE NOTE: Conditionally approved pending the following: 1. Obtaining ALL other relevant approvals. 2. The upload of an updated letter from the Director (current one outdated). 3. UP Survey committee approval letter still to be uploaded

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

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Dear Professor Daniel Nenene Qekwana,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Your application received per email and with the attachments on 4 November 2021, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed by any other act of the Republic of South Africa;
2. The study is approved as per the application and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to MarnaL@Dalrrd.gov.za;
3. All potentially infectious material utilised, collected or generated during the study are to be destroyed at the completion of the study. A registered waste

removal company must dispose the material generated from the study. Records must be kept for five years for auditing purposes;

4. Samples to be used in this study shall not be collected from animals, but from animal handlers and their personal belongings and equipment used in the animal hospital;
5. Bacterial isolation and identification shall be performed at the Veterinary public health (VPH) Laboratory at the University of Pretoria;
6. Pure samples of the microorganisms may be kept under strict access control at the VPH laboratory. Any further usage of the stored samples must be authorised by the Director Animal Health;
7. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 approval.

Title of research/study: Multimodal approaches to reducing transmission of hospital-acquired infections (HAI) in the intensive care unit (ICU) at a veterinary academic hospital.

Researcher:	Ms Dikeledi Carol Sebola
Institution:	University of Pretoria
Our ref Number:	12/11/1/1/MG (2193)
Your ref:	
Expiry date:	31 December 2023

Kind regards,

Name: Dr Mpho Maja
Reason:
Date: 2021.12.17 10:32:12 CAT

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date:

SUBJECT: Multimodal approaches to reducing transmission of hospital-acquired infections (HAI) in the intensive care unit (ICU) at a veterinary academic hospital

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Office of the Registrar

2022-08-19

Ms DC Sebola
Department of Paraclinical Sciences
Faculty of Veterinary Science
University of Pretoria

Email: dcsebola@gmail.com

Dear Ms Sebola

APPROVAL OF RESEARCH STUDY

The UP Survey Coordinating Committee has granted approval for the research study titled "Multimodal approaches to reducing transmission of hospital-acquired infections (HAI) in the intensive care unit (ICU) at a veterinary academic hospital".

The proposed research study has to strictly adhere to the associated study protocol, as well as the UP Survey Policy and the Ethics Committee of the Faculty of Veterinary Science instructions.

Please liaise with the Market Research Office in the Department of Institutional Planning (carlien.nell@up.ac.za) to officially register the study and to finalise the survey regulations, procedures and the fieldwork dates. In order to register the study, the Market Research Office has to receive the formal ethical approval letter from the Faculty of Veterinary Science.

A final electronic copy of the research outcomes must be submitted to the Survey Coordinating Committee as soon as possible after the completion of the study.

Kind regards

Prof CMA Nicholson
REGISTRAR
CHAIRPERSON: SURVEY COORDINATING COMMITTEE

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

1 June 2022

Endorsement Notice

Dear Ms DC Sebola

Ethics Reference No: 187/2022
Title: Multimodal approaches to reducing transmission of hospital-acquired infections (HAI) in the intensive care unit (ICU) at a veterinary academic hospital

The **New Application** as supported by documents received between 2022-04-14 and 2022-06-01 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2022-06-01 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-06-01.
- Please remember to use your protocol number (187/2022) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Fakulteit Geesteswetenskappe
Lefapha la Bomotho

17 May 2022

Dear Ms DC Sebola

Project Title:	Multimodal approaches to reducing transmission of hospital-acquired infections (HAI) in the intensive care unit (ICU) at a veterinary academic hospital
Researcher:	Ms DC Sebola
Supervisor(s):	Prof DN Qekwana
Department:	Paraclinical Sciences
Reference number:	26235286 (REC009-21)
Degree:	Doctoral

I have pleasure in informing you that the above application was **approved** by the Research Ethics Committee on 28 April 2022. Data collection may therefore commence.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should the actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely,

Prof Karen Harris
Chair: Research Ethics Committee
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za