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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 This study aimed to investigate 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 *P. aeruginosa* (48%, 30/62), *A. baumannii* (47%, 29/62), *E. faecium* (35%, 22/62), *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* (23%, 5/22), *P. aeruginosa* (43%, 13/30), *A. baumannii* (24%,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 was reported among just over half (56%, 5/9) 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 belonging to the ESKAPE group of organisms before contact with patients. Moreover, MDR resistance 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, Antimicrobial resistance, Multidrug resistance, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species

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Introduction

Effective hand hygiene has been shown to reduce the transmission of hospital-acquired infections (HAIs) in both human and animal healthcare facilities [1–4]. However, available evidence indicates that hand hygiene compliance among healthcare workers (HCWs) in veterinary medicine remains low [5–7]. This heightens the risk of transmission of infectious diseases and zoonotic organisms within the veterinary hospital setting [2, 8, 9]. 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 [3, 10–12].

Enterococcus faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *and Enterobacter* species (ESKAPE) are the leading cause of HAIs in the intensive care unit (ICU) of both human [13, 14] and animal hospitals [15–17]. Moreover, infections associated with these organisms are less responsive to commonly used antibiotics resulting in limited treatment options and poor patient prognosis, especially in under-resourced developing countries [10–12, 18]. Additionally, some of these organisms can survive in hospital environments for longer, thus remaining a source of infection to susceptible individuals [11, 12, 16].

The intensive care unit (ICU) 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 [12, 19]. Additionally, asymptomatic patients and persons are difficult to identify which also makes them a source of contamination and infection [20]. Hand hygiene compliance remains the most effective strategy to reduce the risk of transmission of organisms associated with HAIs in hospital settings [5, 21, 22]. This study investigated the presence of ESKAPE organisms 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.

Materials and methods

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. The study was done during routine clinical rotations of veterinary students: morning (08h00 to 12h00) and night shifts (20h00 to 08h00).

Study population

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

Sample collection

The study used the glove-juice technique which is well documented in human medicine studies [18, 23]. 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 [24–26]. To sample for the presence of ESKAPE organisms, the dominant hand of each participant was inserted into a sterile latex-free glove containing 20 ml buffered phosphate water (PBW) and massaged for one minute as described by Trick et al. [27] and Matuka et al. [23]. After massaging, the fluid was aseptically retrieved and pipetted into sterile 15 ml tubes then transported on ice within an hour to the veterinary public health (VPH) laboratory of the faculty of veterinary science for further processing.

Screening

Samples brought to the laboratory in PBW were incubated in a shaker at 200 RPM at 37 °C. Since the incubation period was for different bacteria, the time ranged from 16 to 24 h. After enrichment, 100 µl aliquot of the overnight broth was spread on horse blood agar and incubated aerobically at 37 °C for 16–24 h. Following incubation, the plates were assessed for bacterial growth and then prepared for specific bacteria identification using the PCR test.

Identification of ESKAPE bacteria using polymerase reaction chain

DNA extraction

From the 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 [28]. A loopful of the culture sweep was suspended in 1000 µL of sterile FA buffer (BactoTM FA Buffer, Becton and Dickson &Company) in a 1.5 mL Eppendorf tube, vortexed and centrifuged at 12,000 rpm for 5 min. The supernatant

was discarded, and the bacterial pellet was re-suspended in 1000 µL 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 500 µL of sterile distilled water, boiled for 20 min on a heating block, cooled on ice for 10 min, and then stored at -20^{0}C for further processing.

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 targeting specific genes for identifying different bacteria and PCR cycling conditions were used (Table 1). Briefly, for each PCR reaction of 25 µL, the following components were added: 2.5 µL of 10X Thermopol reaction buffer, 2.0 µl of 2.5 mM dNTPs (deoxynucleotide triphosphates), 0.25 µl of 100 mM MgCl2, 1.6 µl of each primer (0.64 µM final concentration), 1U of *Thermus aquaticus* polymerase (Taq) DNA Polymerase (New England BioLabs® Inc.) and 5 µl of DNA lysate template. Positive controls included DNA from the ATCC strains *E. coli* (25922), *S. aureus* (25923), *K. pneumoniae* (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 (0.05 mg/µl) for 15 min, and visualized under ultraviolet (UV) light with a Gel Doc system (Bio-Rad, USA).

Single colony streaking

To differentiate each bacterium, samples that were PCR positive for any of the ESKAPE bacteria during the initial screening were streaked onto differential media 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 MacConkey agar. The plates were then incubated at 37 °C for 16–24 h. Five single colonies of each organism 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 °C for 16–24 h. Following the incubation, genomic DNA was extracted from the colonies, and PCR was performed on the colonies using the same primers as described above to identify them.

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 2) [35].

Antimicrobial resistance testing was performed on Mueller Hinton agar (MHA) (Oxoid, UK) as described by the CLSI [35]. 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 °C for 16–24 h. Each organism was tested against different panels of antibiotics using disks obtained from Oxoid Company as outlined in Table 2. *Escherichia coli* (25922), *S. aureus* (25923), *K. pneumoniae* (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

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

Organism	Gene	Primer sequences (5′-3′)	Amplicon size [a](bp)	Reference
Enterococcus faecium	*sodA*	[b]F: GAAAAAACAATAGAAGAATTAT	215	[29]
		[c]R: TGCTTTTTTGAATTCTTCTTTA		
Staphylococcus aureus	Stpahy-sau	[b]F: AATCTTTGTCGGTACACGATATTCTTCACG	108	[30]
		[c]R: CGTAATGAGATTTCAGTAGATAATACAACA		
Klebsiella pneumoniae	*RcsA*	[b]F: GGATATCTGACCAGTCGG	176	[31]
		[c]R: GGGTTTTGCGTAATGATCTG		
Acinetobacter baumannii	*gryB*	[b]F: CACGCCGTA-AGAGTGCATTA	490	[32]
		[c]R: AACGGAGCTTGTCAGGGTT		
Pseudomonas aeruginosa	16 S rRNA	[b]F: AATACCTTGCTGTTTTGACGTTAC	295	[33]
		[c]R: TCAGTGTCAGTATCAGTCCAGGTG		
Escherichia coli	*gadA*	[b]F: GATGAAATGGCGTTGGCGCAAG	373	[34]
		[c]R: GGCGGAAGTCCCAGACGATATCC		

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

Table 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 (10 µg)	✓		✓		✓	✓
Penicillin-G (10 µg)	✓	✓			✓	
Cefotaxime (30 µg)			✓	✓	✓	✓
Tobramycin (10 µg)			✓	✓	✓	✓
Ciprofloxacin (5 µg)	✓	✓	✓	✓	✓	
Ceftazidime (30 µg)			✓	✓	✓	✓
Ampicillin-sulbactam (10/10µg)			✓	✓	✓	✓
Gentamicin (10 µg)	✓	✓	✓	✓	✓	✓
Imipenem (10 µg)	✓	✓	✓	✓	✓	✓
Trimethoprim-sulfamethoxazole (25 µg)	✓	✓			✓	
Amikacin (30 µg)					✓	
Oxytetracycline (30 µg)		✓	✓	✓		
Erythromycin (15 µg)	✓	✓				
Chloramphenicol (30 µg)	✓	✓				✓
Linezolid (30 µg)		✓				
Oxacillin (1 µg)		✓				
Tetracycline (30 µg)	✓	✓				✓
Total antibiotics	**9**	**11**	**9**	**8**	**11**	**9**

Table 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*)[d]**	**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 (9/9)	56 (5/9)

[b]Antimicrobial resistance, [c]Multidrug resistance, [d] n=number positive for the pathogen, N=total number tested

criteria [35]. However, the intermediate readings were reclassified as resistant for the purpose of data analysis.

Results

Isolated organisms

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 organism and *S. aureus* (24%) was the least identified during the screening. A reduced proportion of isolates were recovered from single colony streaking (Table 3).

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 (89%), cefotaxime (67%), and tobramycin (56%). While two of the three *S. aureus* isolates exhibited resistance to penicillin G (67%). Most *K. pneumoniae* isolates were resistant to ampicillin (86%) and none were resistant to ceftazidime, gentamycin, and imipenem. *Acinetobacter baumannii* isolates exhibited resistance to ampicillin-sulbactam (50%) and one isolate showed resistance to imipenem (25%). All *P. aeruginosa* isolates showed resistance to ampicillin, penicillin-G, and ampicillin-sulbactam, three of the isolates were resistant to imipenem (23%), and two to tobramycin (15%). *Enterococcus faecium* isolates were resistant to penicillin-G (60%) and two (40%) to ciprofloxacin erythromycin, and ampicillin (Table 4).

Multidrug-resistant organisms

Only *E. coli*, *P. aeruginosa*, *E. faecium*, and *S. aureus* had isolates that were resistant to at least one antibiotic in three or more antibiotic classes and thus considered MDR (Table 3).

Discussions

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

Table 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)

the ESKAPE organisms was isolated from the hands of students before entering the ICU. The presence of these organisms is concerning as they are known to cause opportunistic infections and are responsible for most HAIs [11, 12, 36–40]. Moreover, these organisms have zoonotic potential and can be transmitted between humans and animals, posing a health threat to susceptible individuals [16, 40]. The high prevalence of antimicrobial resistance observed among the isolates is also a matter of public health concern. The danger caused by these organisms to public health is exacerbated by the fact that they can adapt and survive in hospital environments [13, 40].

The presence of these organisms on the hands of students before patient contact may indicate that the students are not adhering to hand hygiene compliance measures [5, 41]. Moreover, hand hygiene compliance has been shown to be higher after patient contact suggesting HCWs are more likely to protect themselves rather than the patient [42]. Therefore, hand hygiene compliance must be emphasized at the veterinary academic hospital looking at the five moments of hand hygiene.

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 other studies that reported *E. coli* from the fingertips of HCWs in a human hospital [23] and the hands of HCWs in a veterinary hospital [43]. *Klebsiella pneumoniae* and *E. faecium* were also isolated in this study. A study done in a small animal hospital in Korea [11] also reported the occurrence of these organisms 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, 44]. The presence of these pathogens on environmental surfaces has been associated with faecal contamination [11, 12, 43].

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 [45]. They are among the most prevalent opportunistic organisms in both human and veterinary hospitals [13]. Humans remain important reservoirs for the transmission of these organisms [46]. Similar findings have also been observed by other studies that investigated these organisms from the hands of HCWs [12, 23, 47, 48].

Concerning *P. aeruginosa*, to our knowledge, this is the first study 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 [17, 49]. 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 [23, 46, 50].

Antimicrobial resistance

The resistance in this study was high among the ESKAPE organisms isolated. 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 [51, 52]. Resistance to imipenem in one *A. baumannii* and three *P. aeruginosa* isolates was concerning in this study, given that imipenem is considered a high priority critically important antibiotic by the World Health Organization (WHO) [17, 37, 51, 53]. Regarding trimethoprim-sulfamethoxazole, although the antibiotic may show activity in vitro for *Enterococcus* spp., it is not effective in the treatment of infections associated with these organisms [35]. Notwithstanding, *K. pneumoniae*, *A. baumannii*, and *E. coli* seem sensitive to the ampicillin-sulbactam combination, therefore, may be considered as one of the treatment options.

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 [40, 51, 53]. Ng et al. [54] 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 [12, 52, 55].

Conclusion

Students 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.

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Author contributions
DCS, JWO, MK, and DNQ contributed substantially to the study's conception and design. MNM and DCS were involved in the development of laboratory work protocols. DCS was involved in the acquisition, initial analysis, interpretation of data, and drafting of the article. All the authors 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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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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