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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**Abstract**

**Background:** *Acinetobacter baumannii* and *Klebsiella pneumoniae* are opportunistic bacterial pathogens responsible for hospital-acquired infections 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 (AMR) patterns of *A. baumannii* and *K. pneumoniae* from clinical specimens obtained from dogs presented at a veterinary academic hospital. The findings of this study contribute to an improved understanding of the AMR profile of these bacteria in veterinary medicine.

**Materials and 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. The antimicrobial susceptibility of the isolates was determined using the disk diffusion method following the Clinical and Laboratory Standards Institute guidelines. 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 analyzed 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* isolates (95%, 19/20) 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, and intestinal and lung biopsies. All *K. pneumoniae* 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.

**Keywords:** *Acinetobacter baumannii*, antimicrobial resistance, dogs, ESKAPE, *Klebsiella pneumoniae*, multidrug resistance, veterinary hospital.

**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, and 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 survive for long periods on dry surfaces. 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 from UTIs, bloodstream infections, and...
wound infections in dogs [4, 5]. *Acinetobacter baumannii* associated with hospital-acquired infections is MDR and has a high prevalence of resistance to the β-lactam and cephalosporin groups of antibiotics [4]. The high prevalence of resistance to these groups of antibiotics can be attributed to various reasons, including the natural resistance due to the interplay between the outer membrane that provides protection, active efflux pump systems, and the low 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 diseases, UTIs, pneumonia, bacteremia, 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 to cause clinical mastitis, pneumonia, septicemia, bacteremia, 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, resulting in resistance to β-lactam antibiotics, including penicillin, cephalosporins, and the monobactam aztreonam [6, 9], and, 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 aimed to investigate the antimicrobial resistance (AMR) 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].

**Materials and Methods**

**Ethical approval**

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).

**Study period and location**

The retrospective data were processed in November 2022 and analyzed from January 2023 to April 2023. 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 center 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.

**Data source**

Retrospective data records of dog clinical samples submitted to the Bacteriology Laboratory from January 2007 to December 2013 were used in the study. 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 [12]. Antimicrobial susceptibility testing was performed using the disk diffusion method following Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI 2007, 2008, 2009, 2010, 2011, and 2012) to conduct antimicrobial susceptibility testing.

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

*Klebsiella pneumoniae* isolates were subjected to a panel of 22 antibiotics: amikacin (30 μg), ampicillin (10 μg), carbencillin (100 μg), ceftazidime (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), enrofloxacin (5 μg), erythromycin (15 μg), gentamicin...
isolates (56; 100%) were reported for the various canine samples tested by the bacteriology laboratory at the faculty of veterinary science between 2007 and 2013.

Figure-1: Distribution of Acinetobacter baumannii in K. pneumoniae was also isolated from various samples/tissues such as bone, urine, skin, blood, ear, nasal, and oral cavity (Figure-1).

Antimicrobials to which the bacteria have an inherent resistance were excluded from MDR analysis. For example, K. 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 lincomamides, 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. Therefore, imipenem, tobramycin, rifampin, 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.

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 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.

Results

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 baumannii was also isolated from various samples/tissues such as bone, urine, skin, blood, ear, nasal, and oral cavity (Figure-1).

Nineteen isolates were AMR (95%, 19/20) with the majority of A. baumannii isolates showing resistance to penicillin G (85%) and ampicillin (65%). Forty-five percentages (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 against 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-1).

Sixty percentages (60%, 12/20) of A. baumannii isolates were MDR. A high proportion of isolates exhibited resistance to cephalothin (92%), followed by chloramphenicol, trimethoprim-sulphamethaxazole, enrofloxacin, amoxicillin/clavulanic acid, and kanamycin, to which 75% of the isolates were resistant (Table-1). Three (n = 3) MDR A. baumannii isolates were resistant to ten antimicrobials, two (n = 2) to nine antimicrobials, and one (n = 1) to eight antimicrobials (Table-2).

Klebsiella pneumoniae

A total of 56 K. pneumoniae isolates were recorded. Of these, 39% (22/56) were isolated from urine 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 biopsies, and lung biopsies (Figure-2).

All K. pneumoniae isolates (56; 100%) were resistant to penicillin G, amoxicillin, carbenicillin, piperacillin, ceftazidime, and lincomycin. Sixty-four percentages (35/56) of the isolates were resistant to cephalexin and 60% to amoxicillin/clavulanic acid. None of the isolates tested were resistant to
Table-1: Antimicrobial resistance and multidrug resistance profile of Acinetobacter baumannii isolated from canine clinical samples tested at a veterinary academic hospital, in South Africa.

<table>
<thead>
<tr>
<th>Antimicrobial category</th>
<th>Isolates (n) %</th>
<th>MDR isolates (n) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tylosine</td>
<td>68 (13/19)</td>
<td>-</td>
</tr>
<tr>
<td>β-lactams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>65 (13/20)</td>
<td>-</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100 (5/5)</td>
<td>-</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>85 (17/20)</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>100 (5/5)</td>
<td>-</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>100 (5/5)</td>
<td>-</td>
</tr>
<tr>
<td>Cefalothin/lexin</td>
<td>60 (12/20)</td>
<td>92 (11/12)</td>
</tr>
<tr>
<td>Combination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>45 (9/20)</td>
<td>75 (9/12)</td>
</tr>
<tr>
<td>Carbapenem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>25 (1/4)</td>
<td>-</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>30 (6/20)</td>
<td>50 (6/12)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>20 (4/20)</td>
<td>33 (4/12)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>47 (9/19)</td>
<td>75 (9/12)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>80 (4/5)</td>
<td>-</td>
</tr>
<tr>
<td>Lincosamides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lincomycin</td>
<td>95 (19/20)</td>
<td>-</td>
</tr>
<tr>
<td>Lincomycin-spectinomycin</td>
<td>60 (12/20)</td>
<td>-</td>
</tr>
<tr>
<td>Potentiated sulfas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulphamethoxazole</td>
<td>45 (9/20)</td>
<td>75 (9/12)</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>40 (8/20)</td>
<td>67 (8/12)</td>
</tr>
<tr>
<td>Orbifloxacin</td>
<td>45 (9/20)</td>
<td>75 (9/12)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>35 (7/20)</td>
<td>50 (6/12)</td>
</tr>
<tr>
<td>Amphenicol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>60 (9/15)</td>
<td>75 (9/12)</td>
</tr>
</tbody>
</table>

MDR=Multidrug-resistant

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

<table>
<thead>
<tr>
<th>Patterns</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI_CEP_CHL_OXY_ENR_GEN_KAN_ORB_SUL_SYN</td>
<td>3</td>
</tr>
<tr>
<td>AMI_CEP_CHL_OXY_ENR_GEN_KAN_ORB_SUL_SYN</td>
<td>2</td>
</tr>
<tr>
<td>CEP_CHL_SYN</td>
<td>2</td>
</tr>
<tr>
<td>CEP_CHL_ENR_GEN_KAN_ORB_SUL_SYN</td>
<td>1</td>
</tr>
<tr>
<td>CEP_OXY_ENR_ORB_SUL_SYN</td>
<td>1</td>
</tr>
<tr>
<td>CEP_ChL_KAN_ORB_SUL_SYN</td>
<td>1</td>
</tr>
<tr>
<td>AMI_CEP_KAN_SUL</td>
<td>1</td>
</tr>
<tr>
<td>ENR_KAN_SUL</td>
<td>1</td>
</tr>
</tbody>
</table>

Total=12

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

Discussion

This study investigated the AMR 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. pneumoniae were isolated from various clinical samples. This confirms past research findings that reported these organisms as associated with various clinical infections in dogs [5, 7, 14–17]. Moreover, these organisms have been associated with nosocomial infections and can disseminate resistance genes to other bacteria [18, 19]. Cleaning and disinfection of the environment have proven effective in reducing the burden of these organisms in the environment [20]. However, these organisms can persist in a dry environment and continue to be a source of infection to susceptible patients [14, 20, 21]. Therefore, careful monitoring of dogs admitted to the veterinary hospital through routine surveillance is important to prevent the transmission of these pathogens between patients.

Antibiotic resistance patterns of A. baumannii

Antibiotic resistance among A. baumannii isolates is increasing and is associated with increased morbidity, mortality, and treatment costs in the intensive care unit [22]. 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 academic hospital in South Africa.

A low prevalence of resistance to imipenem among *A. baumannii* has been reported in a study by Pailhòries et al. [14]. In this 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].

\textit{Acinetobacter baumannii} was resistant to trimethoprim-sulphamethoxazole, which is consistent with findings in other studies [27, 28]. This could be due to the overproduction of or alteration in plasmid-mediated dihydrofolate reductase associated with trimethoprim resistance [29]. Although \textit{A. baumannii} exhibited resistance to trimethoprim-sulphamethoxazole, evidence suggests that it should be considered for uncomplicated infections [28, 30, 31].

Resistance to aminoglycosides among \textit{A. baumannii} was generally not common in this study, with the exception of tobramycin. This was expected given that resistance to tobramycin among \textit{A. baumannii} increased [32, 33], mainly associated with the synthesis of aminoglycoside-modifying enzymes (AME) and efflux pump systems [32, 34]. This finding has significant public health implications, given that aminoglycosides are commonly used to treat \textit{A. baumannii} infections. Therefore, trends in the susceptibility of these organisms should be monitored [25, 32].

Fluoroquinolones are generally used to treat \textit{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 antibiotics and is mediated by efflux-mediated quinolones resistance [26, 35–37]. Therefore, care is needed to prevent misuse and overuse of fluoroquinolones to curb the development of resistance [38, 39]. A low prevalence of resistance to oxytetracycline was also observed in this study. This is encouraging due to the potential use of tetracyclines as monotherapy or in combination with other antimicrobials for the treatment of \textit{A. baumannii} infections [27, 40, 41].

Forty-five percentages (n = 5; 45%) of \textit{A. baumannii} isolates were MDR. However, a higher prevalence of \textit{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 \textit{A. baumannii} is not uncommon [42]. Given this, available evidence suggests that the choices for treatment of MDR \textit{A. baumannii} infections may include carbapenems, colistin, and combination antimicrobials [4, 25, 32, 43].

\textbf{Antibiotic resistance patterns of \textit{K. pneumoniae}}

Similar to the study conducted in South Korea [20] and France [44], most \textit{K. pneumoniae} isolates in this study were resistant to \(\beta\)-lactam antimicrobials. The \(\beta\)-lactam resistance among \textit{K. pneumoniae} isolates is attributed to the production of the plasmid-mediated sulphhydryl variable-1 a penicillinase [9, 17, 44–46]. However, none of the \textit{K. pneumoniae} isolates in this study exhibited resistance to carbapenems. This is consistent with the findings of Haenni et al. [44] in a study conducted in France. These findings suggest that carbapenem could be considered as a treatment option for \textit{K. pneumoniae} [46, 47].

The prevalence of resistance to aminoglycosides varied in this study. For example, low resistance was observed to amikacin and gentamycin [48, 49], while high resistance was observed to tobramycin and kanamycin. The varying prevalence of resistance among aminoglycosides could be attributed to the different resistance mechanisms. For example, resistance to amikacin and gentamycin is associated with the presence of enzymatic modification enzymes (AME) and/or 16S ribosomal RNA methyltransferase (16S-RMTases) [3, 48, 50], whereas tobramycin resistance is associated with the presence of the aminoglycoside N-acetyltransferases (6’)-Ib(-like) protein and not AME or 16S-RMTase genes [51]. Despite the nephrotoxicity of aminoglycosides [52], this group of antimicrobials has been used effectively in the treatment of \textit{K. pneumoniae} infections in both human and veterinary medicine [3].

Consistent with findings from both human and animal studies [16, 17, 46], resistance to enrofloxacin, and orbifloxacin among \textit{K. pneumoniae} isolates was low in this study. Similar to other studies, resistance to trimethoprim-sulphamethoxazole was observed in this study [17, 36]. The low resistance in this study is encouraging, as trimethoprim-sulphamethoxazole is the drug of choice in the treatment of UTIs [17, 53]. In addition, trimethoprim-sulphamethoxazole is effective in the treatment of patients with carbapenemase-producing \textit{K. pneumoniae} infections [54].

Almost all \textit{K. pneumoniae} isolates in this study were MDR. This is not unusual, as AMR genes are frequently observed in this organism [17]. What is of concern is that the role of companion animals as reservoirs for human infections associated with resistant \textit{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.

\textbf{Limitation}

The data used in this study were limited to one veterinary hospital and excluded 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.

\textbf{Conclusion}

\textit{Acinetobacter baumannii} and \textit{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 AMR among \textit{A. baumannii} and \textit{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 overemphasized.

**Authors’ Contributions**

All authors were involved in the study design. DCS: Involved in the data collection and data management, performed all statistical analyses, interpreted the results, and prepared the draft manuscript. DNQ: Involved in data analysis and interpretation of the results, as well as extensive revision of the manuscript. JWO: Extensively reviewed and edited the manuscript. MMK: Involved in the editing of the manuscript. All authors have read, reviewed, and approved the final manuscript.

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**Competing Interests**

The authors declare that they have no competing interests.

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