

Prevalence of Multi-Drug Resistance (MDR) in ESKAPE Pathogens from Different Sources in Lagos, Nigeria

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Abstract

ESKAPE pathogens are six multidrug-resistant bacteria that pose serious public health risks and are difficult to treat. This study evaluated the prevalence of ESKAPE pathogens and antimicrobial resistance profiles. A cross-sectional study was conducted in Lagos, collecting 500 samples from clinical (n = 350), environmental (n = 100), and animal (n = 50) sources between December 2023 and February 2024. The samples were analyzed, and standard methods were used for antibiotic susceptibility testing. RT-PCR was used to detect resistance (*bla*_{SHV}, *bla*_{TEM}, *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{IMP}) and virulence gene (*ompA*, *toxA*, *magA*, *gelE*) markers. A prevalence of 7.6% (38/500) ESKAPE pathogens was recorded, with source rates of 5.4% clinical, 10% animal, and 14% environmental samples. *Pseudomonas aeruginosa* was 100% resistant to cefepime and ceftazidime and 92.3% to vancomycin. *Klebsiella pneumoniae* exhibited 100% resistance to cefepime, and *Enterococcus faecium* was 100% resistance to vancomycin. *Staphylococcus aureus* and *Enterobacter* spp. were resistant to multiple antibiotics. ESKAPE pathogens showed 28 antibiotic resistance patterns, with *P. aeruginosa* predominantly exhibiting CIP-VA-AUG-CAZ-CPM-MEM-FOX pattern. Four isolates from clinical samples, two each from *Acinetobacter baumannii* and *P. aeruginosa* produced AmpC. ESKAPE pathogens were multidrug-resistant, with over 70% showing a Multiple Antibiotic Resistance (MAR) index greater than 0.5. *Acinetobacter baumannii* strain harboured extended-spectrum beta-lactamases (ESBL) and carbapenemase genes, while an environmental *K. pneumoniae* strain had the *bla*_{OXA-48} gene and the virulence genes *ompA*, *magA*, and *toxA*. The study underscores the urgent need for improved surveillance and targeted strategies to combat these resistant ESKAPE pathogens and their gene expression across different sources.

Keywords: ESKAPE, pathogens, antimicrobial resistance, virulence genes

Резюме

Патогените ESKAPE са шест мултирезистентни бактерии, които представляват сериозни рискове за общественото здраве и са трудни за лечение. Това проучване оценява разпространението на патогените на ESKAPE и профилите на антимикробна резистентност. В Лагос е проведено напречно проучване, като са събрани 500 проби от клинични (n = 350), екологични (n = 100) и животински (n = 50) източници между декември 2023 г. и февруари 2024 г. Пробите са анализирани и са използвани стандартни методи за тестване на чувствителност към антибиотици. Използвана е RT-PCR за откриване на маркери за резистентност (*bla*_{SHV}, *bla*_{TEM}, *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{IMP}) и гени за вирулентност (*ompA*, *toxA*, *magA*, *gelE*). Регистрирана е разпространеност от 7.6% (38/500) на патогени на ESKAPE, с процент на източник от 5.4% клинични, 10% животински и 14% екологични проби. *Pseudomonas aeruginosa* е 100% резистентен към цефепим и цефотаксим и 92.3% към ванкомицин. *Klebsiella pneumoniae* показва 100% резистентност към цефепим, а *Enterococcus faecium* е 100% резистентен към ванкомицин. *Staphylococcus aureus* и *Enterobacter* spp. са резистентни към множество антибиотици. ESKAPE патогените показват 28 модела на антибиотична резистентност, като *P. aeruginosa* предимно проявява CIP-VA-AUG-CAZ-CPM-MEM-FOX модел. Четири изолата от клинични проби, по два от *Acinetobacter baumannii* и *P. aeruginosa*, продуцират AmpC. ESKAPE патогените са мултирезистентни, като над 70% показват индекс на множествена антибио-

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тична резистентност (MAR) по-голям от 0,5. Щамът *Acinetobacter baumannii* е притежавал гени за бета-лактамази с широк спектър (ESBL) и карбапенемази, докато щам на *K. pneumoniae* от околната среда е имал гена blaOXA-48 и гените за вирулентност *ompA*, *magA* и *toxA*. Проучването подчертава неотложната необходимост от подобро наблюдение и целенасочени стратегии за борба с тези резистентни ESKAPE патогени и тяхната генна експресия в различни източници.

Introduction

The Centers for Disease Control and Prevention (CDC) has stated that ESKAPE bacteria pose a significant global health threat due to their potential to raise morbidity and mortality rates in infected individuals (Loyola-Cruz *et al.*, 2023). These pathogens can lead to longer hospital stays and higher costs for both patients and the healthcare system. (Catalano *et al.*, 2023). In 2008, the term ESKAPE was introduced to describe a group of pathogens that can “escape” the lethal effects of antibiotics. (Rice, 2008). The pathogens include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, which are among the leading causes of potentially fatal infections, and it is widely acknowledged that they can develop and transmit antibiotic resistance (Santajit and Indrawattana, 2016; Mulani *et al.*, 2019). Furthermore, to guide the development of new antibiotics, the World Health Organization (WHO) recently established a global priority list of antibiotic-resistant pathogens. Among the critical priorities is the *Enterobacterales* order, which includes several antibiotic-resistant organisms, such as those resistant to third-generation cephalosporins (Shrivastava *et al.*, 2018; Benkö *et al.*, 2020). As a result, the entire *Enterobacterales* group is often categorized within the ESKAPE group of bacteria in various publications (Denissen *et al.*, 2022). Despite a recent rise in awareness of the issue, there is a lack of data on the prevalence of healthcare-associated infections (HCAIs) in veterinary medicine compared to human medicine (Stull and Weese, 2015). Nevertheless, the increasing importance of animal healthcare-associated infections (HCAIs) in modern veterinary practice, infection control measures are still in their early stages (Walther *et al.*, 2017). This is particularly true for companion animals, such as dogs, cats, and horses, where growing research has documented nosocomial outbreaks of various etiologies. These outbreaks are often complicated by the zoonotic and antibiotic-resistant traits of the involved microorganisms (Walther *et al.*, 2017). The presence of ESKAPE pathogens in the environment can be attributed to various human activities, such as swimming, bathing, improper hospital waste disposal, sewage spills, and

agricultural runoff (Hrenovic *et al.*, 2017; Amarasiri *et al.*, 2020). Additionally, these pathogens have been found in beach sand and intertidal waters (Akanbi *et al.*, 2017). Industrial and municipal wastewater systems act as reservoirs for ESKAPE pathogens, which aid in their environmental spread (Gwenzi, 2020). Soils affected by human activities and dumping sites can also contain these pathogens, promoting their transmission and persistence (Amarasiri *et al.*, 2020). Furthermore, ESKAPE pathogens can contaminate vegetables (Ebomah and Okoh, 2020), raw or prepared foods (Verraes *et al.*, 2013), and water sources, including irrigation, groundwater, and drinking water systems, posing risks to both animal and human health (Ebomah and Okoh, 2020). For these reasons, this study aims to investigate the prevalence of multidrug-resistant ESKAPE pathogens in humans, animals, and the environment, including food, in Lagos, Nigeria.

Materials and Methods

Study design, place, duration

This cross-sectional study was done to assess the prevalence of ESKAPE pathogens from different sources in Lagos, Nigeria. A total of 500 samples were collected from different sources, including clinical, abattoir and environmental, from December 2023 to February 2024.

Samples collection

Clinical samples, including oral swabs, wound swabs, sputum, and urine, were randomly collected from patients involved in this study at two prominent hospitals in Lagos State: Lagos State University Teaching Hospital (LUTH) and Badagry General Hospital (BGH). The samples were sourced from individuals aged eighteen and older, of both genders, who had been hospitalized for at least 14 days. In total, the study comprised 350 patient samples collected aseptically by laboratory scientists from these facilities. Additionally, 50 samples of hospital effluent and Mazamaza Lagoon were gathered from various locations on different days at the lagoon and two hospitals. At Cele Abattoir, Otto Awori and Oko Oba, veterinary doctors, collected 100 cow rectal swabs using sterile swab sticks.

Sample processing and identification of ESKAPE isolates

A total of 200 urine samples underwent visual assessment for quality, emphasizing colour, clarity, and any particulate matter. Turbid samples or those with visible sediment were centrifuged to concentrate potential pathogens. Approximately 5 ml of 50 blood samples were obtained from patients showing clinical signs of chronic septicemia. The samples were obtained and placed in a sterile container with 50 ml of thioglycolate broth, maintaining a blood-to-broth ratio of 1:10. Blood culture bottles were incubated at 37°C and checked daily after 48 hours for 5 days. Indicators of microbial growth included turbidity, hemolysis, gas formation, or colour changes. If no growth was detected within 7 days, the result was considered negative. Upon visible growth, a small amount of broth was aseptically withdrawn and subcultured. Forty sputum samples were homogenized to ensure even pathogen distribution. Fifty wound swab samples and ten oral swabs were aseptically collected with sterile forceps and placed in sterile saline for the elution of the collected material. These samples were initially cultured on selective agars, such as MacConkey (HIMEDIA, Mumbai, India), Bile aesculin (Tintan media, Rajasthan, India) from Hardy Diagnostics, Mannitol salt (HiMedia Laboratories Pvt. Ltd., India) and Cetrimide agar (HiMedia Laboratories Pvt. Ltd., India). Colonies of interest were then further cultured on Blood agar and Muller Hinton agar to assess hemolysis and pigmentation, with all plates incubated at 37°C for 18-24 hours for growth evaluation. Isolate identification was conducted using standard bacteriological techniques, focusing on morphology, colony characteristics, hemolysis, and pigment production. Additionally, 50 hospital effluent samples, each comprising 100 ml of water from various sites, were collected and mixed with 90 ml of Tryptone Soya Broth, then incubated aerobically at 37°C for 24 hours. A 100 µL aliquot of the bacterial suspension was streaked onto selective agar plates, which were incubated aerobically at 37°C for 24-48 hours. Animal samples were collected with sterile swabs and sent to the Microbiology Research Laboratory at Lagos State University, where isolates were identified following the methods outlined (Chika *et al.*, 2018). Additional identification techniques included Gram staining and various biochemical tests, such as the Triple Sugar Iron (TSI) test, catalase test, oxidase test, citrate utilization, indole production, and urea hydrolysis (Yang *et al.*, 2021).

Antimicrobial susceptibility testing

Susceptibility testing was conducted using the disc diffusion method following the Clinical and Laboratory Standards Institute Guidelines. Antibiotic discs from Mast (Mast Diagnostics, Merseyside, UK) were utilized, including cefepime (30µg), augmentin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), imipenem (10 µg), colistin sulfate (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and tigecycline (15 µg). Results were interpreted by measuring the diameters of inhibition zones. When VET01S breakpoints were not available, human breakpoints were used, and the diameter zones of inhibition were evaluated according to the guideline's performance standards. The control strain used for the testing was *Escherichia coli* ATCC 25922. To identify multidrug-resistant ESKAPE pathogens, organisms that showed resistance to at least three different classes of antibiotics were selected (Bayot and Bragg, 2024).

Phenotypic identification of AmpC beta-lactamase (AmpC) and extended-spectrum beta-lactamase (ESBL)

The identification of ESBL-producing isolates was carried out phenotypically using the double disc synergy test (El Aila *et al.*, 2023). This method involved inoculating a test organism on a Mueller-Hinton agar (Oxoid, U.K.) plate. Four antibiotic discs, ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), and amoxicillin/clavulanic acid (20/10 µg), were positioned 20 mm apart from the central amoxicillin/clavulanic acid disc, ensuring that their edges were in contact. After 24 hours of incubation, a positive result was indicated by an increased zone of inhibition between any of the cephalosporin antibiotics and the amoxicillin/clavulanic acid disc. This enhanced inhibition zone suggested a synergistic effect with clavulanic acid, confirming the presence of an ESBL-producing organism (El Aila *et al.*, 2023).

For evaluating AmpC production, the AmpC Disk Test was performed as described by Black *et al.* (2025). In-house filter paper disks were prepared by mixing 20 µl saline with 100x Tris-EDTA, dried, and stored at low temperatures (2 - 8°C). A lawn of cefoxitin-susceptible *E. coli* strain ATCC 25922 was spread on a Mueller-Hinton agar plate. Just before testing, the AmpC disks were rehydrated with 20 µl of saline, and a few colonies of the test organisms (ESKAPE isolates) were applied to one of these disks. A disk containing 30 µg of cefoxitin was placed on the agar surface, face down, with the AmpC disk positioned close to it. The inverted agar

plates were incubated overnight at 35°C in ambient air. After incubation, the plates were inspected for results. A positive result was indicated by an indentation or flattening of the inhibition zone around the cefoxitin disk, while a negative result showed no distortion (Black *et al.*, 2005).

DNA extraction from ESKAPE pathogens

DNA was extracted from bacterial colonies grown on the selective media using bacterial lysis buffer (Tris-hydroxymethylaminomethane and NaCl) and heat treatment. These were carried out using the Luna Universal qPCR protocol (New England BioLabs), following the manufacturer's instructions. The process involved mechanical disruption using a bead beater for 25 min, followed by centrifugation of the ZR Bashing Bead Lysis Tube at 10,000× g for 1 min. β-Mercaptoethanol was added to the Genomic Lysis Buffer (final concentration: 0.5%) to enhance performance. Pure ESKAPE isolate cells were placed into the lysis tube with the Bashing Bead Buffer and centrifuged at high speed for 15 min. The supernatant was filtered through a Zymo-Spin II-F Filter (Zymo Research, Irvine, CA, USA) at 8000× g for 1 min, then washed and centrifuged with a Zymo-Spin ICR Column. The DNA was then eluted by adding DNA Elution Buffer to the column and centrifuging at 10,000× g for 3 s, resulting in ultra-pure DNA. The supernatant containing the extracted DNA was collected

and stored at −20°C (Ajoseh *et al.*, 2025). The RT-PCR Rotor-Gene Q 2plex (Qiagen, Hilden, Germany) amplification was performed using specific primer sets for resistance (*bla_{SHV}*, *bla_{TEM}*, *bla_{KPC}*, *bla_{OXA-48}*, and *bla_{IMP}*) and virulence (*ompA*, *toxA*, *magA* and *gelE*) genes (Table 1), with PCR mixtures prepared using specific volumes and concentrations. The PCR process entailed 45 cycles, each consisting of a denaturation phase at 95°C for 30 s, followed by an annealing step at 50°C for 30 s, and concluding with an extension at 72°C for 60 s (Akinyemi *et al.*, 2021).

Statistical analysis

In this study, the collected data were analyzed using IBM SPSS software (version 20). The results were displayed in various formats, including frequency tables, charts, and written descriptions. Key variables were cross-tabulated, and the Chi-square test was used to evaluate the relationships between variables. The Multiple Antibiotic Resistance Index (MARI) is a simple metric calculated as the number of antibiotics to which a bacterial isolate is resistant divided by the total number of antibiotics tested.

Resultsw

A total of 500 samples from different sources, clinical (n=350), environmental (n=100) and animal (50) were examined. An overall prevalence of 7.6% (38/500) of tested samples was positive

Table 1. Primer sets for virulence and resistance genes

Genes	Primer Sequence (5 ¹ – 3 ¹)	References
<i>bla_{SHV}</i>	F – CGCCTGTGTATTATCTCCCT R – CGAGTAGTCCACCAGATCCT	(Ungo-Kore <i>et al.</i> , 2019)
<i>bla_{TEM}</i>	F – TTTCGTGTCGCCCTTATTCC R- ATCGTTGTCAGAAGTAAGTTGG	(Ungo-Kore <i>et al.</i> , 2019)
<i>bla_{KPC}</i>	F- CGTCTAGTTCTGCTGTCTTG R- CTTGTTCATCCTTGTTAGGCG	(Booq <i>et al.</i> , 2022)
<i>bla_{OXA-48}</i>	F – GCGTG GTTAAGGATGAACAC R- CATCAAGTTCAACCCAACCG	(Gondal <i>et al.</i> , 2024)
<i>bla_{IMP}</i>	F- GGAATAGAGTGGCTTAAYTCTC R – GGTTTAAYAAAACAACCACC	(Yamakawa <i>et al.</i> , 2019)
<i>ompA</i>	F: GTTAAAGGCGACGTAGACG R: CCAGTGTTATCTGTGTGAC	(Abdulhussein and Mahdi, 2023)
<i>gelE</i>	F-TATGACAATGCTTTTGGGAT R-AGATGCACCCGAAATAATAT	(Vankerckhoven <i>et al.</i> , 2004)
<i>toxA</i>	F- CTGCGCGGGTCTATGTGCC R – GATGCTGGACGGGTCGAG	(Mahmood, 2023)
<i>magA</i>	F – GGTGCTCTTTACATCATTGC R- GCAATGGCCATTTGCGTTAG	(Mohammed and Flayyih, 2018)

Table 2. Biochemical characterization of isolates from different sources

lant	Gram stain	Butt	Gas	H ₂ S	Lactose	Citrate	Urease	Indole	Catalase	Oxidase	Suspected organism
Alkali	-	Alkali	-	-	-	+	-	-	+	+	<i>P. aeruginosa</i>
Acid	-	Alkali	+	-	+	+	+	-	+	-	<i>K. pneumonia</i>
Alkali	+	Alkali	-	-	+	+	-	-	-	+	<i>S. aureus</i>
Alkali	+	Acid	-	-	+	-	-	-	-	-	<i>E. faecium</i>
Alkali	-	Alkali	-	-	-	+	-	-	+	-	<i>A. baumannii</i>
Acid	-	Alkali	+	-	-	+	+	-	+	-	<i>Enterobacter spp</i>

Key: H₂S: Hydrogen sulphide, +: positive -: negative

for ESKAPE pathogens, including 0.4% (2/500) *E. faecium*, 0.4% (2/500) *S. aureus*, 1.8% (9/500) *K. pneumoniae*, 2.2% (11/500) *A. baumannii*, 2.6% (13/500) *P. aeruginosa*, and 0.2% (1/500) *Enterobacter spp*. The identification of these pathogens was conducted using cultural, morphological, and biochemical methods (Table 2).

The frequency distribution of positive isolates from different sources is presented in Table 3. In clinical samples, 5.4% (19/350) were found positive for ESKAPE pathogens. Of the 19 ESKAPE from clinical samples, 0.6% (11/19) were isolated from males and 0.5% (8/19) were female subjects. *P. aeruginosa* was the most frequently isolated pathogen, with 5 strains from males and 3 from females' gender in this study. ESKAPE pathogen was mostly implicated in the age group 50 years and above, 0.6% (12/19), with *P. aeruginosa* and *A. baumannii* being the most prominent. Animal samples showed a positive rate of 10% (5/50), while environmental samples had a positive rate of 14% (14/100) for ESKAPE pathogens (Figure 1 and Table 3).

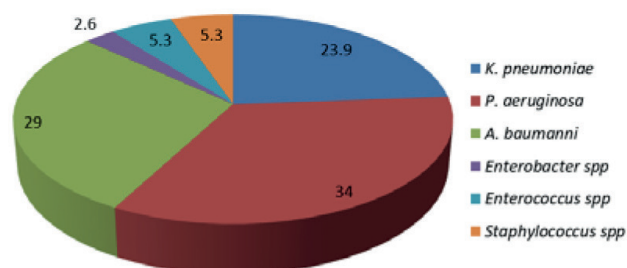


Fig. 1. Percentage of ESKAPE isolates from the positive samples reviewers'

The results of AST show that all the ESKAPE pathogens identified in this study were multiple drug resistant, with over 70% of these pathogens exhibiting a MAR index of >0.5 (Table 4). Specifically, 2 strains of *A. baumannii*, BLS9 and URS6 from blood and urine, respectively, exhibited a 0.7

MAR index. Similarly, 5 strains of *P. aeruginosa* BLS4, BLS5, BLS6, BLS8 and WOS7 were observed to have a MAR index of 0.7 and were isolated from clinical samples.

Three strains of *K. pneumoniae* (ENL1, ENE8 and ENE2) were isolated from environmental samples with a higher MAR index of 0.6, and then strain BLS10 from the clinical sample showed a MAR index of 0.5, the two strains of *E. faecium* ARS2 and ARS3 exhibited a MAR index of 0.6 similarly, *S. aureus* strains URS1 and URS6 have MAR index of 0.7 and 0.9 respectively and were all isolated from cattle. Nevertheless, a strain of *Enterobacter spp*. from a urine sample had a low MAR index of 0.3 with 100% susceptibility to gentamicin, and imipenem and completely resisted augmentin, cefoxitin, ceftazidime, cefepime, imipenem, meropenem and vancomycin (Table 4). Furthermore, *P. aeruginosa* strains are 100% resistant to cefepime and cefoxitin, 92.3% to vancomycin and 69.2% to augmentin. *K. pneumoniae* strains were 100% resistant to cefepime, and 66.7% to cefoxitin and vancomycin. *E. faecium* developed 100% resistance to vancomycin and augmentin, with 50% susceptibility to gentamicin, ciprofloxacin and carbapenem antibiotics. *S. aureus* strains were 100% resistant to 7 different antibiotics: augmentin, cefoxitin, ceftazidime, cefepime, imipenem, meropenem and vancomycin. Furthermore, all the ESKAPE isolates exhibited 95% susceptibility to gentamicin and ciprofloxacin (Table 5). The 38 ESKAPE pathogens exhibited 28 antibiotic resistance patterns altogether and CIP-VA-AUG-CAZ-CPM-MEM-FOX was the most frequently encountered pattern exhibited by 4 strains of *P. aeruginosa* BLS1, BLS4, BLS6 and WOS7. It was followed by VA-CPM-FOX-IMI exhibited by 3 ESKAPE isolates, one from *P. aeruginosa* and 2 from *K. pneumoniae*, while other ESKAPE isolates exhibited one or two of the resist-

Table 3. Bacterial pathogens isolated from different sources in Lagos, Nigeria by sample source, age and sex

Category		Variables	No. of Sam- ples	No. of + samples n (%)	No of + ESKAPE Pathogens n (%)					
Subcategory					<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>K. pneumoniae</i>	<i>E. faecium</i>	<i>S. aureus</i>	<i>Enterobacter spp.</i>
Clinical	Hospital	BGH	150	14 (9.3)	7 (4.7)	6 (4)	1 (0.7)	0 (0)	0 (0)	0(0)
		LASUTH	200	4 (2)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	2 (1)	1(0.5)
		Total	350	19 (5.4)	8 (2.3)	7 (2)	1 (0.3)	0 (0)	2 (0.6)	1(0.3)
		Urine	200	4(2)	0 (0)	1 (0.5)	0 (0)	0 (0)	2 (1)	1(0.5)
		Blood	50	14 (28)	7 (14)	6 (12)	1 (2)	0 (0)	0 (0)	0 (0)
		Sputum	40	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Wound	50	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Oral swab	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Total	350	19 (5.4)	8 (2.3)	7 (2)	1 (0.3)	0 (0)	2 (0.6)	1(0.3)
	Sex	Male	207	11 (5.3)	5 (2.4)	4 (1.9)	0 (0)	0 (0)	2 (1)	0 (0)
		Female	143	8(4.9)	3 (2.1)	3 (2.1)	1 (0.7)	0 (0)	0 (0)	1(0.7)
		Total	350	19 (5.4)	8 (2.3)	7 (2)	1 (0.3)	0 (0)	2 (0.6)	1(0.7)
	Age	18 – 30	50	1 (2)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
		31 – 49	100	6 (5)	3 (3)	2 (2)	0 (0)	0 (0)	0 (0)	1(0.1)
		> 50	200	12 (6)	5 (2.5)	4 (2)	1 (0.5)	0 (0)	2 (1)	0 (0)
		Total	350	19 (5.4)	8 (2.3)	7 (2)	1 (0.3)	0 (0)	2 (0.6)	1(0.1)
Environmental	Effluent	Ok o ba abattoir	10	2 (20)	0 (0)	0 (0)	2 (20)	0 (0)	0 (0)	0 (0)
		Cele abattoir	10	2 (20)	0 (0)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Lagoon water	BGH	20	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		LASUTH	20	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Total	40	10 (25)	3 (7.5)	1 (2.5)	6 (15)	0 (0)	0 (0)	0 (0)
Animal	Study area	Ok o ba	5	1 (20)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Cele abatto	20	1 (0)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Oto Awori	25	3 (12)	0 (0)	1 (4)	0 (0)	2 (8)	0 (0)	0 (0)
		Total	50	5 (10)	2 (4)	1 (2)	0 (0)	2 (4)	0 (0)	0 (0)
	Samples collected	Nasal Swab	10	2 (20)	1 (10)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)
		Rectal Swab	40	3 (7.5)	1 (2.5)	0 (0)	0 (0)	2 (5)	0 (0)	0 (0)
		Total	50	5 (10)	2 (4)	1 (2)	0 (0)	2 (4)	0 (0)	0 (0)
		Grand total	500	38 (7.6)	13 (2.6)	11(2.2)	9 (1.8)	2 (0.4)	2 (0.4)	1 (0.2)

Key: LASUTH: Lagos State University Teaching Hospital, BGH: Badagry General Hospital, NS: Nasal swab, RS: Cattle Rectal swab, (%): percentage n= number

Table 4. Antibiotics Susceptibility Test (AST) of ESKAPE pathogens Isolated

Isolated Pathogens	Strain code	Sample source	Location	Antibiotics Used										No. of Res	No. of Sen	MAR
				GM	PTZ	CIP	VA	AUG	CAZ	CPM	MEM	FOX	IMI			
<i>A. baumannii</i>	BLS47	Blood	Badagry	S	S	S	R	S	R	R	R	R	R	6	4	0.6
	BLS12	Blood	BGH	S	S	S	R	S	S	R	R	R	R	5	5	0.5
	BLS31	Blood	BGH	S	S	S	R	R	R	R	R	S	S	5	5	0.5
	BLS15	Blood	BGH	S	S	S	R	R	S	R	R	R	S	5	5	0.5
	BLS9	Blood	BGH	S	S	R	R	R	R	R	S	R	R	7	3	0.7
	BLS38	Blood	BGH	S	S	S	R	R	R	R	S	R	R	6	4	0.6
	URS6	Urine	Lasuth	S	R	R	R	S	R	R	R	R	R	8	2	0.7
	ANS4	Cattle N S	OAA	S	S	S	R	S	R	R	R	R	S	5	5	0.5
	ENE3	CeleA E	CA	S	S	S	S	S	R	S	S	S	S	1	9	0.1
	ENE5	CeleA E	CA	S	S	S	S	S	R	R	S	S	S	2	8	0.2
<i>P. aeruginosa</i>	ENL4	Lagoon	ML	S	S	S	R	S	R	S	S	R	S	3	7	0.3
	BLS4	Blood	BGH	S	S	R	R	R	R	R	R	R	S	7	3	0.7
	BSL1	Blood	BGH	S	S	R	R	R	R	R	R	R	S	7	3	0.7
	BLS25	Blood	BGH	S	S	R	R	S	R	R	R	R	S	6	4	0.6
	BLS6	Blood	BGH	S	S	R	R	R	R	R	R	R	S	7	3	0.7
	BLS5	Blood	BGH	S	S	R	R	R	R	R	S	R	R	7	3	0.7
	BLS38	Blood	BGH	S	S	S	R	S	S	R	S	R	R	4	6	0.4
	BLS12	Blood	BGH	S	S	S	R	R	R	R	S	R	S	5	5	0.5
	WOS7	Wound	Lasuth	S	S	R	R	R	R	R	R	R	S	7	3	0.7
	ANS3	Cattle N S	OOA	S	S	S	R	R	S	R	S	R	R	5	5	0.5
<i>K. pneumoniae</i>	ARS3	Cattle N S	OOA	S	S	S	R	S	R	R	R	R	S	5	5	0.5
	ENL2	Lagoon	ML	S	S	S	R	R	S	R	R	R	S	5	5	0.5
	ENL7	Lagoon	ML	S	S	S	S	S	R	R	S	R	S	3	7	0.3
	ENL1	Lagoon	ML	S	S	R	R	R	R	R	S	R	S	6	4	0.6
	BLS5	Blood	BGH	S	S	S	R	R	R	R	S	S	R	5	5	0.5
	ENE5	Effluent	OOA	S	S	R	S	S	S	R	R	S	S	3	7	0.3
	ENE8	Effluent	OOA	S	S	S	R	R	R	R	R	R	S	6	4	0.6
	ENL22	Lagoon	ML	S	S	S	R	S	S	R	S	R	R	4	6	0.4
	ENL2	Lagoon	ML	S	S	R	R	R	R	R	S	R	S	6	4	0.6
	ENL8B	Lagoon	ML	S	S	S	S	S	S	R	R	R	S	3	7	0.3
<i>E. feacium</i>	ENL13	Lagoon	ML	S	S	S	S	R	S	R	S	R	S	3	7	0.3
	ENL11A	Lagoon	ML	S	S	S	R	R	S	R	R	S	S	4	6	0.4
	ENL19	Lagoon	ML	S	S	S	R	S	S	R	S	R	R	4	6	0.4
	ARS 2	Cattle R S	OA	S	S	R	R	R	-	S	R	-	R	5	3	0.6
	ARS3	Cattle R S	OA	R	R	S	R	R	-	R	S	-	S	5	3	0.6
<i>S. aureus</i>	URS9	Urine	Lasuth	S	S	S	R	R	R	R	R	R	R	7	3	0.7
	URS6	Urine	Lasuth	R	S	R	R	R	R	R	R	R	R	9	1	0.9
<i>Enterobacter spp</i>	URS8	Urine	Lasuth	S	S	S	R	R	R	R	R	R	S	3	7	0.3

KEY: GM = Gentamicin 30 µg, PTZ = Piperacillin tazobactam 110 µg, CIP = Ciprofloxacin 5 µg, VA = Vancomycin 5 µg, AUG = Augmentin 30 µg, CAZ = Ceftazidime 10 µg, CPM = Cefpiramide 30µg, MEM = Meropenem 10 µg, FOX = Cefixitin 30 µg, IMI = Imipenem 10 µg; BLS: Blood sample; URS= Urine sample; WOS = Wound swab; ANS = Animal nasal swab; ARS = Animal rectal swab; ENE= Environmental effluents; ENL= Environmental lagoon water

Table 5. Antimicrobial susceptibility and resistance test on isolates

Class of antibiotics	Antibiotics	<i>A. baumannii</i>		<i>P. aeruginosa</i>		<i>K. pneumoniae</i>		<i>E. faecium</i>		<i>S. aureus</i>		<i>Enterobacter</i> spp	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Aminoglycoside	Gentamycin	0 (0)	11 (100)	0 (0)	13 (100)	0 (0)	9 (100)	1 (50)	1 (50)	1 (50)	1 (50)	0(0)	1(100)
	Piperacillin tazobactam	1 (9.1)	10 (90.9)	0 (0)	13 (100)	0 (0)	9 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0(0)	1(100)
Fluroquinolone	Ciprofloxacin	2 (18.2)	9 (81.8)	7 (53.8)	6 (46.2)	2 (22.2)	7 (77.8)	1 (50)	1 (50)	1 (50)	1 (50)	0(0)	1(100)
Penicillin	Augmentin	4 (36.4)	7 (63.4)	9 (69.2)	4 (30.8)	5 (55.6)	4 (44.6)	1 (50)	1 (50)	2 (100)	0 (0)	0(0)	1(100)
Cephalosporin (2nd - 4th generation)	Cefoxitin (2nd)	9 (81.8)	2 (18.2)	13 (100)	0 (0)	6 (66.7)	3 (33.3)	1 (50)	1 (50)	2 (100)	0 (0)	100(0)	0(0)
	Ceftazidim (3rd)	9 (81.8)	2 (18.2)	10 (76.9)	3 (23.1)	3 (33.3)	6 (66.7)	2 (100)	0 (0)	2 (100)	0 (0)	100(0)	0(0)
	Cefepime (4th)	10 (90.9)	1 (9.1)	13 (100)	0 (0)	9 (100)	0 (0)	1 (50)	1 (50)	2 (100)	0 (0)	100(0)	0(0)
Carbapenem	Imipenem	5 (45.5)	6 (54.5)	3 (23.1)	10 (76.9)	3 (33.3)	6 (66.7)	1 (50)	1(50)	2 (100)	0 (0)	100(0)	0(0)
	Meropenem	6 (54.5)	5 (45.5)	7 (53.8)	6 (46.2)	4 (44.6)	5 (55.6)	1 (50)	1 (50)	2 (100)	0 (0)	100(0)	0(0)
Glycopeptide	Vancomycin	9 (81.8)	2 (18.2)	12 (92.3)	1 (7.7)	6 (66.7)	3 (33.3)	2 (100)	0 (0)	2 (100)	0 (0)	0(0)	1(100)

Table 6. Antibiotic resistance pattern and resistance profile of ESKAPE pathogens

Isolated Pathogens	Strain code	Sample source	Location	Resistance Pattern	Resistance profile
<i>A. baumannii</i>	BLS47	Blood	Badagry	VA-CAZ-CPM-MEM-FOX-IMI	F
	BLS12	Blood	BGH	VA--CPM-MEM-FOX-IMI	M
	BLS31	Blood	BGH	VA-AUG-CAZ-CPM-MEM	N
	BLS15	Blood	BGH	VA-AUG-CPM-MEM-FOX	O
	BLS9	Blood	BGH	CIP-VA-AUG-CAZ-CPM-FOX-IMI	C
	BLS38	Blood	BGH	VA-AUG-CAZ-CPM-FOX-IMI	G
	URS6	Urine	Lasuth	PTZ-CIP-VA-CAZ-CPM-MEM-FOX	B
	ANS4	Cattle N S	OAA	VA-CAZ-CPM-MEM-FOX	P
	ENE3	CeleA E	CA	CAZ	ZB
	ENE5	CeleA E	CA	CAZ-CPM	ZA
	ENL4	Lagoon water	ML	VA-CAZ-FOX	V
	BLS4	Blood	BGH	CIP-VA-AUG-CAZ-CPM-MEM-FOX	D
<i>P. aeruginosa</i>	BSL1	Blood	BGH	CIP-VA-AUG-CAZ-CPM-MEM-FOX	D
	BLS25	Blood	BGH	CIP-VA-CAZ-CPM-MEM-FOX	H
	BLS6	Blood	BGH	CIP-VA-AUG-CAZ-CPM-MEM-FOX	D
	BLS5	Blood	BGH	CIP-VA-AUG-CAZ-CPM-FOX-IMI	C
	BLS38	Blood	BGH	VA-CPM-FOX-IMI	T
	BLS12	Blood	BGH	VA-AUG-CAZ-CPM-FOX	Q
	WOS7	Wound	Lasuth	CIP-VA-AUG-CAZ-CPM-MEM-FOX	D
	ANS3	Cattle N S	OOA	VA-AUG-CPM-FOX-IMI	R
	ARS3	Cattle N S	OOA	VA-CAZ-CPM-MEM-FOX	P
	ENL2	Lagoon water	ML	VA-AUG-CPM-MEM-FOX	O
	ENL7	Lagoon water	ML	CAZ-CPM-FOX	W
	ENL1	Lagoon water	ML	CIP-VA-AUG-CAZ-CPM-FOX	I
<i>K. pneumoniae</i>	BLS5	Blood	BGH	VA-AUG-CAZ-CPM-IMI	S
	ENE5	Effluent	OOA	CIP-CPM-MM	X
	ENE8	Effluent	OOA	VA-AUG-CAZ-CPM-MEM-FOX	J
	ENL22	Lagoon water	ML	VA-CPM-FOX-IMI	T
	ENL2	Lagoon water	ML	CIP-VA-AUG-CAZ-CPM-FOX	I
	ENL8B	Lagoon water	ML	CPM-MEM-FOX	Y
	ENL13	Lagoon water	ML	AUG-CPM-FOX	Z
	ENL11A	Lagoon water	ML	VA-AUG-CPM-MEM	U
	ENL19	Lagoon water	ML	VA-CPM-FOX-IMI	T
<i>E. faecium</i>	ARS 2	Cattle R S	OA	CIP-VA-AUG-MEM-FOX-IMI	K
	ARS3	Cattle R S	OA	GM-PTZ-VA-AUG=CAZ-CPM	L
<i>S. aureus</i>	URS9	Urine	Lasuth	VA-AUG-CAZ-CPM-MEM-FOX-IMI	E
	URS6	Urine	Lasuth	GM-CIP-VA-AUG-CAZ-CPM-MEM-FOX-IMI	A
<i>Enterobacter spp</i>	URS8	Urine	Lasuth	VA-AUG-CAZ-CPM-MEM-FOX	J

Key: BLS: Blood sample; URS= Urine sample; WOS = Wound swab; ANS = Animal nasal swab; ARS = Animal rectal swab; ENE= Environmental effluents; ENL= Environmental lagoon water

ance patterns (Table 6). Of the 38 ESKAPE isolates, 4 isolates comprising 2 strains each of *A. baumannii* and *P. aeruginosa* from clinical samples were found to produce AmpC phenotypically. The plate for the AmpC-positive isolate is shown in Fig. 2.

Seven ESKAPE isolates, mainly 4 strains of *A. baumannii*, were CAB positive, of which 2 strains (BLS12 and BLS4) were from a clinical source and one strain each from animal (ARS3) and

lagoon water (ENL4), respectively. The remaining 3 isolates were *P. aeruginosa* BLS14, BLS38 and BLS5 strains from blood samples. Similarly, seven isolates were ESBL producers; these included mainly *S. aureus* URS6, *A. baumannii* BLS12, BLS6, *E. faecium* ARS 2, ARS 3, and *K. pneumoniae* ENL2 and ENL3. It is interesting to note that a strain from *A. baumannii* (BLS6) was found to be an AmpC and ESBL producer. *A. baumannii* BLS12

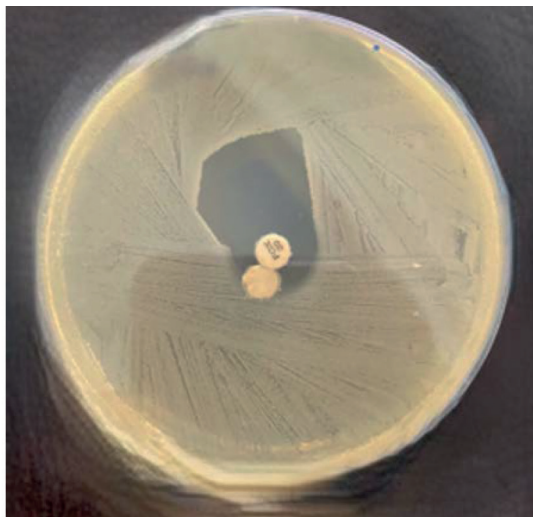


Fig. 2. The image depicts a positive Modified Hodge Test result, indicated by the characteristic cloverleaf-like enhanced growth of the *E. coli* indicator strain along the test organism's streak toward the carbapenem disk, suggesting positive AmpC production

produces both CAB+ and ESBL (Table 7). Notably, among the ESKAPE pathogens in clinical isolates, only one *A. baumannii* strain carried multiple resistance genes (bla_{SHV} , bla_{TEM} , bla_{KPC} , bla_{OXA-48} , and bla_{IMP}). Additionally, a single *A. baumannii* strain from animals contained the bla_{SHV} gene, while a *K.*

pneumoniae strain from environmental isolates had the bla_{OXA-48} gene. The *ompA* virulence gene was present in all *A. baumannii* strains, the *toxA* gene was found in clinical *P. aeruginosa* isolates, and the *magA* gene was detected in two environmental *K. pneumoniae* strains (Table 7).

Discussion

The ESKAPE bacteria, as identified by the CDC and WHO, pose a significant global health challenge due to their potential impact on morbidity and mortality (CDC, 2019; WHO, 2017). These bacterial pathogens can lead to prolonged hospital stays and escalate healthcare costs for both the health system and patients due to their ability to resist or “ESKAPE” multiple antibiotics (WHO, 2017). In this study, the overall prevalence of ESKAPE pathogens isolated from different sample sources is 7.6% (38/500), made up of five genera, namely: *E. faecium* (2), *S. aureus* (2), *K. pneumoniae* (9), *A. baumannii* (11), *P. aeruginosa* (13) and *Enterobacter* spp (1) isolated from clinical, animal, and environmental sources. However, the source prevalence of pathogens in this study showed that 5.4% (19/350), 10% (5/50), and 14% (14/100) were clinical, animal, and environmental sources,

Table 7. Phenotypic detection of MDR, ESBL, CAB and AmpC producing ESKAPE and genotypic detection of specific virulence and resistance genes

Parameters	Isolates	Strain code	Resistance gene							Virulence gene				
			AmpC +	CAB +	ESBL +	<i>Bla</i> _{SHV}	<i>Bla</i> _{TEM}	<i>Bla</i> _{IMP}	<i>Bla</i> _{OXA-48}	<i>Bla</i> _{KPC}	<i>ompA</i> .	<i>tox</i> A,	<i>mag</i> A	<i>gel</i> E
Clinical	<i>S. aureus</i>	URS6	-	-	-	-	-	-	-	-	-	-	-	-
	<i>A. baumannii</i>	BLS12	-	+	-	+	+	+	+	+	+	-	-	-
		BLS4	+	+	-	-	-	-	-	-	-	-	-	-
		BLS6	+	-	+	-	-	-	-	-	-	-	-	-
	<i>P. aeruginosa</i>	BLS1	-	+	-	-	-	-	-	-	-	-	-	-
		BLS38	+	+	-	-	-	-	-	-	-	+	-	-
		BLS5	-	+	-	-	-	-	-	-	-	-	-	-
		WOS7	+	-	-	-	-	-	-	-	-	+	-	-
Animal	<i>Enterobacter</i> spp	URS8	-	-	-	-	-	-	-	-	-	-	-	-
	<i>E. faecium</i>	ARS2	-	-	-	-	-	-	-	-	-	-	-	-
		ARS3	-	-	-	-	-	-	-	-	-	-	-	-
	<i>A. baumannii</i>	ANS4	-	+	-	+	-	-	-	-	+	-	-	-
Environmental	<i>K. pneumoniae</i>	ENL2	-	-	+	+	+	-	-	-	-	-	+	-
		ENL3	-	-	+	+	+	-	-	-	-	-	+	-
	<i>A. baumannii</i>	ENL4	-	+	-	-	-	-	-	-	+	-	-	-

Key: ESBL: Extended Spectrum Beta Lactamase, CAB: Carbapenemase, AmpC: Ampicillin beta lactamase; BLS: Blood sample; URS= Urine sample; WOS = Wound swab; ANS = Animal nasal swab; ARS = Animal rectal swab; ENE= Environmental effluents; ENL= Environmental lagoon water.

respectively. These results indicated that ESKAPE pathogens are more commonly found in the environment than in other sources. Zaheer *et al.* reported more ESKAPE pathogens in environmental sources, such as effluent and wastewater, than clinical or animal sources; their result is consistent with the present study (Zaheer *et al.*, 2020). A similar study conducted by Savin *et al.* (2020) documented *K. pneumoniae*, *A. baumannii* and *S. aureus* as the most frequently isolated ESKAPE pathogens in wastewater and processed water from German slaughterhouses (Savin *et al.*, 2020).

The distribution of 19 ESKAPE pathogens from clinical samples reveals that *P. aeruginosa* was the most prevalent, with 8 (42%) isolates identified in blood, urine and wound samples, followed by 7(36.8%) *A. baumannii*, 2(10.5%) *S. aureus*, 1(5.3%) *K. pneumonia* and 1(5.3%) *Enterobacter* spp. Similarly, Zhong and He (2021) recorded 16% *K. pneumoniae*, 14% *A. baumannii*, and 9.8% *P. aeruginosa* from hospitalized patients attending general hospitals in Guilin, China (Zhong and He, 2021). However, this contrasts with the findings of Benkő *et al.* (2020), who reported only two ESKAPE pathogens - *K. pneumoniae* (13.4%) and *S. aureus* (11.3%) from hospitalized patients in Hungary (Benkő *et al.*, 2020) and with 14.5% *Enterobacter* spp reported in a study on multidrug-resistant rates in clinical isolates in Iran (Mirzaei *et al.*, 2021). This variation in the distribution of these pathogens might be associated with disparity in geographical location, sample sources and other environmental factors. Of the 19 ESKAPE from clinical samples, 11(57.8%) were isolated from males and 7(42.1%) from female subjects. *P. aeruginosa* was the most frequently isolated pathogen, with 5 strains from males and 3 from females' gender. ESKAPE pathogen was mostly implicated in the age group 50 years and above, 63% (12/19), with *P. aeruginosa* and *A. baumannii* most prevalent.

This study corroborated El-Kady *et al.*, who recorded more strains of ESKAPE pathogens in male patients attending a tertiary hospital in Jeddah, Saudi-Arabia, than female counterparts, with *P. aeruginosa*, *S. aureus* and *A. baumannii* being the most predominant ESKAPE pathogens (El-Kady *et al.*, 2022). Furthermore, among patients aged 60 years or older, the occurrence of MDR ESKAPE strains was 71.1%, while non-MDR strains accounted for 58.0%. (El-Kady *et al.*, 2022). This might be connected to the activities of male patients, and the immune status is more likely to be hospitalized, where exposure to multidrug-resist-

ant pathogens is higher. However, Alsharedeh *et al.* found contrasting results in their study on urinary tract infections among Jordanian patients. They reported that 92% of ESKAPE pathogens were detected in female subjects, while only 8% were found in males. Most were prevalent in the age -group of 21-30 years old. (Alsharedeh *et al.*, 2023). The observed findings can be attributed to anatomical factors, which include the relatively short length of the urethra, the proximity of the urethral meatus to the anus, sexual activity, the absence of prostatic secretions, pregnancy, and susceptibility to contamination from gastrointestinal and faecal flora as previously reported (Pardeshi, 2018; Kaur and Kaur, 2021). It may also be associated with behavioural practices such as incontinence, and suboptimal toilet habits or the combination of both anatomical and behavioural factors (Pardeshi, 2018; Kaur and Kaur, 2021).

In this study, 5 (10.0%) of the ESKAPE isolates, notably 2(4%) each of *E. faecium* and *P. aeruginosa*, and 1(2%) *A. baumannii*, were recovered from the rectal swab of cattle. The occurrence of these pathogens in cattle has been frequently reported by different authors. For example, Singh (2018) reported the six ESKAPE pathogens in animals associated with various disease conditions, particularly in cattle (Singh, 2018). These ESKAPE isolates recorded high percentage of *Enterobacter* species (44.4%), followed by *K. pneumoniae* (22.1%), *P. aeruginosa* (16.6%), *S. aureus* (12.98%), *E. faecium* (3.6%), and *A. baumannii* (0.23%). Although, no *Enterobacter* species was isolated in the present study, the varying prevalence and vast occurrence of ESKAPE pathogens in his study compared to the current work might be connected to the sample size, the clinical prognosis of the animals investigated, and other factors capable of influencing pathogenesis and immune response in animals (De Oliveira *et al.*, 2020). Furthermore, of the 14% (14/100) ESKAPE pathogens recovered from environmental samples in this study, 8(8.0%) *K. pneumoniae*, 3(3.0%) *P. aeruginosa* and 3(3.0%) *A. baumannii* were obtained. This finding shared some similarity with the report of Savin *et al.*, who documented that the majority of the recovered ESKAPE bacterial isolates (94.9%, n = 448/472) from German poultry slaughterhouse wastewater and process water consisted primarily of the *A. calcoaceticus* - *A. baumannii* (ACB) complex (32.4%), followed by (12.3%) *S. aureus* and (10.8%) *K. pneumoniae*. However, *Enterobacter* spp., *Enterococcus* spp., and *P. aeruginosa* constituted a

smaller proportion (5.1%) of the isolates (Savin *et al.*, 2020). Similarly, Phoon *et al.* reported varying occurrences of ESKAPE pathogens in the environment of a tertiary hospital in Malaysia (Phoon *et al.*, 2018). The slight disparity in the frequency of ESKAPE pathogens observed in this study with other studies might be connected to environmental conditions, and the specific context of sample collection (e.g., wastewater vs. hospital environments) can significantly influence the diversity and abundance of ESKAPE pathogens. Nevertheless, the environment remains the reservoir of these bacterial pathogens, serving as a vehicle of transmission to humans and animals (Iwu and Okoh, 2019).

The *in vitro* antimicrobial susceptibility testing results indicated that all five members of the ESKAPE pathogens identified in this study exhibited multidrug resistance, with over 70% demonstrating a significant MAR index of greater than 0.5. For instance, *A. baumannii* 10(90.9%) were resistant to cefepime, 9(81.8%) to ceftazidime and ceftazidime, while 6(54.5%) to meropenem. Specifically, two strains of *A. baumannii*, BLS9 and URS6 from blood and urine samples, respectively, exhibited a MAR index of 0.7 to CIP, VA, AUG, CAZ, CPM, FOX, IMP and PTZ, CIP, VA, CAZ, CPM, MEM and FOX antibiotics, respectively. Raheem and Al-Hasnawy (2020) highlighted significant resistance (100%) of *A. baumannii* to cephalosporins, specifically, ceftazidime, cefepime, and cefotaxime. Similarly, investigations carried out in Iraq by Alsehlawi *et al.* (2014) and AL-kadhmi (2015) revealed that *A. baumannii* exhibited complete resistance to the same cephalosporins.

Additionally, Lowings *et al.* (2020) found that all MDR-*Acinetobacter baumannii* isolates were 100% resistant to cefotaxime, 89% to ceftazidime, and 90% to cefepime (Lowings *et al.*, 2015). In a similar vein, Anane and colleagues observed a significant proportion of *A. baumannii* strains resistant to both imipenem (81%) and meropenem (83%). Furthermore, Yadav *et al.* (2015) and Shrestha *et al.* (2020) reported elevated levels of *A. baumannii* resistance against meropenem, with rates of 89.4% and 50%, respectively. Additionally, a study conducted by He *et al.* (2011) in China revealed that 85% of isolates exhibited carbapenem resistance, a finding that agreed with the results of this study. These findings underscore the critical importance of vigilant surveillance and effective management strategies in combating antimicrobial resistance. Similarly, of the 13 *P. aeruginosa* strains isolated from different sources, all (100%) were resistant to

cefepime and ceftazidime, 12 (92.30%) to vancomycin, 10 (76.9%) to ceftazidime, 9(69.2%) to augmentin, 7(53.8%) to ciprofloxacin, and 7(53.8%) to meropenem. Specifically, five strains of *P. aeruginosa* - BLS4, BLS1, BLS6, BLS5 and WOS7 isolated from clinical samples were observed to have a MAR index of 0.7. This finding is similar to that of Japoni *et al.* (2006) who reported 84.3% *P. aeruginosa* strains resistant to ceftazidime, 72.9% to ciprofloxacin and 30% to meropenem.

The high occurrence of ceftazidime resistance could potentially be attributed to several factors, such as widespread utilization of drugs, the extensive administration of a specific antibiotic leading to selective pressure on susceptible isolates, and transferable genetic elements, such as plasmids or transposons. This assertion has been well documented in the literature (Carmeli *et al.*, 1999). Also, all the 9 strains of *K. pneumoniae* identified in this study were resistant to cefepime, 6 (66.7%) to ceftazidime and vancomycin, 5 (55.6%) to augmentin, 4 (44.6%) to meropenem, 3 (33.3%) to ceftazidime and imipenem. Specifically, three strains of *K. pneumoniae* (ENL1, ENE8 and ENL2) from environmental samples revealed a high MAR index of 0.6, while strain BLS10 from a clinical sample exhibited a MAR index of 0.5. This study shared some similarities with the report of those who observed *K. pneumoniae* with reduced sensitivity to SXT, CIP, GM, and MEM (Arbune *et al.*, 2021). Interestingly, the two strains of *E. faecium*, ARS2 and ARS3, demonstrated a multidrug resistance index of 0.6. All the *E. faecium* strains were resistant to vancomycin, with 50% (1/2) resistant to all other antibiotics investigated in this study. This corresponds with the result of Roy *et al.* (2022), who reported all the *E. faecium* isolates in their study to be resistant to ampicillin, and frequently (93.33%–55.56%) resistant to ceftriaxone, cefotaxime, streptomycin, erythromycin, and imipenem; moderate to lower (26.67%–4.44%) resistance to tetracycline, ciprofloxacin, norfloxacin, chloramphenicol, gentamicin, and vancomycin.

Interestingly, 80% of *E. faecium* strains isolated from healthy broiler chickens in Bangladesh were MDR (95% CI: 66.18%–89.10%) (Roy *et al.*, 2022). The presence of resistance genes from *E. faecium* in animals is of significant public health concern (Urmi *et al.*, 2021). These genes can be transmitted to humans through contaminated food supply chains or close contact with the animals, potentially leading to the emergence of MDR and multiple antibiotic-resistant (MAR) bacteria in

both human and animal populations (Urmi *et al.*, 2021; Roy *et al.*, 2022). The finding from this study was further corroborated by Salami *et al.*, where *E. faecium* showed 100% resistance to ciprofloxacin, erythromycin, imipenem, vancomycin, meropenem, and ampicillin in cattle rectal swabs in Lagos, Nigeria (Salami *et al.*, 2025). All *S. aureus* strains isolated in this study were 100% resistant to augmentin, cefoxitin, ceftazidin, cefepime, imipenem, meropenem and vancomycin. The two strains, URS9 and URS6, isolated from patients with urinary tract infections, displayed MAR indices of 0.7 and 0.9, respectively. This is consistent with the finding of Gashe *et al.*, who reported that isolated strains of *S. aureus* (ESKAPE pathogens) showed 23.4% (11/47) and 34% (16/47) resistance to ceftriaxone and ceftazidime, respectively (Gashe *et al.*, 2018).

Furthermore, the *Enterobacter* strain in this study was completely susceptible to gentamicin and imipenem, while exhibiting complete resistance to augmentin, cefoxitin, ceftazidime, cefepime, meropenem, and vancomycin. This complete susceptibility is consistent with findings in multiple studies. For example, a study by Kowalski *et al.* (2024) reported high susceptibility rates for gentamicin in *Enterobacter* spp. isolates, often around 90-95% in clinical settings. This was also corroborated by the growing trend of ESBL production in *Enterobacter* spp. reported by El-Ruz *et al.* (2025) highlighting that many *Enterobacter* isolates exhibit resistance to these antibiotics, particularly in urinary tract infections (UTIs). Generally, gentamicin and piperacillin-tazobactam were the most effective antibiotics against all the ESKAPE pathogens isolated in this study, as most of the ESKAPE pathogens were susceptible to both. This is against the report of Slavcovici *et al.* (2015) who documented high resistance of ESKAPE isolates against gentamicin and moderate resistance to piperacillin-tazobactam. Nevertheless, the most effective antibiotics globally are piperacillin-tazobactam and aminoglycosides (Zohar *et al.*, 2020). However, the basis for these differences might be connected to pressure of antibiotics, sample sources, types, geographical locations and other environmental factors. A study conducted by McManus (1997) highlighted that many bacteria have become resistant to multiple classes of antibacterial agents through genetic exchange mechanisms, and these MDR bacteria have become a cause of serious concern, especially in hospitals and other healthcare units where they tend to develop resistance to the most commonly used first-line

antibiotics.

It is noteworthy that of the 38 ESKAPE isolates recovered from this study, 3 (8.1%) were ESBL and 5 (13.5%) carbapenemase producers, while 4 (10.8%) were AmpC-producing isolates. Of the ESBL producers, 1 (2.7%) was *A. baumannii*, and 2 (7.4%) *K. pneumoniae* were isolated from different sources. Furthermore, this study reported that 2(5.4%) each of *A. baumannii*, and *P. aeruginosa* were carbapenemase-producing strains. This is slightly consistent with Founou *et al.* (2018), who reported MDR *K. pneumoniae* and *Enterobacter* spp. as emerging pathogens in hospital settings. This observation aligns with findings from a South African study, where *K. pneumoniae* was identified as the primary pathogen in stool samples from children residing in the community of KwaZulu-Natal, South Africa (Mahomed and Coovadia, 2015). Similarly, an elevated prevalence of ESBL-producing *Enterobacteriaceae* was demonstrated among Egyptian patients with community-acquired gastrointestinal complaints. This was further corroborated by Singh (2018), who reported varying occurrences of ESBL and carbapenemase-producing strains of ESKAPE pathogens among sick animals (Singh, 2018). Intriguingly, resistance to beta-lactams, particularly cephalosporins and carbapenems, exhibited via ESBL, AmpC and carbapenemase production in bacterial pathogens is closely linked to elevated mortality rates and cross-resistance to other antibiotic classes, including cephalosporins and quinolones (Yadav *et al.*, 2020). The circulation of ESKAPE pathogens in clinical and veterinary settings in Lagos, Nigeria, necessitates further research on the genetic basis of ESBL, AmpC and carbapenemase-producing ESKAPE pathogens, and a nuanced approach to combating this formidable threat in our ecosystem.

Furthermore, this study revealed that *A. baumannii* is of significant concern among ESKAPE pathogens, particularly due to its harbouring of multiple resistance genes, including *bla*_{SHV}, *bla*_{TEM}, *bla*_{KPC}, *bla*_{OXA-48}, and *bla*_{IMP}. This is consistent with studies that have highlighted *A. baumannii* as a major nosocomial multidrug-resistant pathogen in clinical settings (Vázquez-López *et al.*, 2020; Ibrahim *et al.*, 2021). Furthermore, it could be a result of healthcare-associated infections capable of acquiring resistance mechanisms through horizontal gene transfer (Nguyen and Joshi, 2021). The detection of the *bla*_{SHV} resistance gene in a strain of *A. baumannii* from animal isolates raises interesting questions about the transmission dynamics of re-

sistant strains between humans and animals. Prior studies have suggested that while *A. baumannii* is prevalent in clinical environments; its presence in animal isolates is less common, which may reflect differences in exposure or selective pressures in various environments (Pailhoriès *et al.*, 2015; Nocera *et al.*, 2021). Interestingly, ESBL genes (*bla*_{SHV} and *bla*_{TEM}) were found in two strains of *K. pneumoniae* in the environmental isolates. This is similar to the report by Mondal *et al.*, where environmental strains of *K. pneumoniae* frequently carry *bla*_{SHV} and *bla*_{TEM}, highlighting their role in antibiotic resistance (Mondal *et al.*, 2019). However, this finding does not align with the report by Akinyemi *et al.*, that clinical isolates of *K. pneumoniae* contained both *bla*_{CTX-M-I} and *bla*_{SHV} genes, indicating ESBL production, but none had the *bla*_{TEM} gene (Akinyemi *et al.*, 2021).

The absence of resistant genes in other ES-KAPE strains in environmental isolates aligns with findings from other research, indicating that environmental reservoirs may not be significant sources of resistance genes for these pathogens (Denissen *et al.*, 2022). This further highlights the need for careful monitoring of clinical and animal isolates to prevent the spread of resistance. Furthermore, the presence of the virulence gene *ompA* in all strains of *A. baumannii* across all sources supports previous findings that *ompA* is critical for the pathogenicity of this organism (Marazzato *et al.*, 2022). Conversely, the detection of the *tox**A* virulence gene in *P. aeruginosa* from clinical isolates in this study confirms its role in virulence, as established in numerous studies linking *tox**A* to the severity of infections caused by this bacterium (Nikbin *et al.*, 2012; Chand *et al.*, 2021). Furthermore, the virulence gene *magA* was also detected in two strains of *K. pneumoniae* isolated from environmental sources. This finding aligns with a study that the *magA* gene associated with the K1 capsule serotype was found in 19.2% of isolates, with no significant variation among clinical specimens (Kot *et al.*, 2023). The presence of virulent *K. pneumoniae* strains in this study poses a transmission risk to humans via contaminated water, soil, or food, especially in areas with poor sanitation.

Conclusions

In conclusion, this study reveals a concerning prevalence of ES-KAPE pathogens in Lagos, Nigeria, with a notable 7.6% positive samples across the clinical, environmental, and animal sources. The high rates of multidrug resistance among these pathogens highlight a significant public health chal-

lenge, particularly with *P. aeruginosa* and *S. aureus* exhibiting complete resistance to multiple antibiotics. It is worth noting that *A. baumannii* and *K. pneumoniae* were significant contributors to ESBL production, and *P. aeruginosa* in carbapenemase production. The presence of virulence genes, such as *ompA* in *A. baumannii*, *tox**A* in *P. aeruginosa* and *magA* in *K. pneumoniae*, alongside the detection of resistance genes in clinical and environmental isolates, highlights the pathogenic potential of these organisms and the risk of transmission through contaminated environments. The data underscore the urgent need for improved surveillance and targeted strategies to combat these resistant bacteria. Enhanced infection control measures and antibiotic stewardship to mitigate the impact of these pathogens on public health and ensure effective treatment options.

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Ethical consideration

The ethical approval for sample collection was granted by the Lagos State Teaching Hospital Ethics Committee, with reference number LREC/06/10/2518.

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