RESEARCH ARTICLE

REVISED Phenotypic and genetic extended spectrum beta lactamase profiles of bacterial isolates from ICU in tertiary level hospital in Kenya

[version 2; peer review: 2 approved]

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Abstract

Background

Bacterial infections in the Intensive Care Units are a threat to the lives of critically ill patients. Their vulnerable immunity predisposes them to developing bacteria-associated sepsis, deteriorating their already fragile health. In the face of increasing antibiotics resistance, the problem of bacterial infection in ICU is worsening. Surveillance of bacterial infections in ICUs and drug resistance will help to understand the magnitude of the problem it poses and inform response strategies. We assessed bacterial infections in ICU setting by identifying prevalent Gram-negative bacterial species and characterized their antibiotic susceptibility patterns.

Methods

Cross-sectional samples collected from Kenyatta National Hospital ICU between January and June 2021 were cultured and phenotypic identification of culture-positive samples performed using VITEK 2. Antibiotic susceptibility patterns were determined based on

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Antimicrobial Susceptibility Testing (AST) results. Cephalosporin-resistant Gram-negative bacteria were assessed by PCR to detect the presence of ESBL genes including (*bla* CTX-M, *bla* SHV, *bla* TEM, *bla* OXA)

Results and discussion

Out of the 168 Gram-negative isolates, *Acinetobacter baumanii* was the most abundant (35%). Other isolates that were present at frequencies more than 15% are *Klebsiella pneumoniae* and *Escherichia. coli. A. baumaniii* is known to be a notorious bacterium in ICU due to its multidrug resistance nature. Indeed, *A. baumanii* isolates from Kenyatta National Hospital showed significantly high level of phenotypic resistance. Concordant with the high level of phenotypic resistance, we found high carriage of the ESBL genes among the isolates analysed in this study. Moreover, majority of isolates harboured all the four ESBL genes.

Conclusion

A high rate of phenotypic and genetic resistance was detected among the tested isolates. Resistance to cephalosporins was primarily driven by acquisition of the ESBL genes. The high prevalence rate of ESBL genes in ICU bacterial isolates shown in this study has a important implication for ICU patient management and general antibiotics use.

Keywords
Intensive Care Unit, anti-biotic susceptibility, gram-negative, cephalosporins, Extended Spectrum Beta Lactamase

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REVISED **Amendments from Version 1**

This version of the manuscript has gone through a rigorous review, addressing the first and second reviewer comments. However, methods used, and data generated remains essentially the same. We have added the significance of the study in the background section of the abstract and corrected the names of the genes tested in our study. We have done minor edits throughout the background section. We have also made minor edits in the methods section; the subtitle "AST and phenotypic detection" has been revised to "Antimicrobial Susceptibility Testing (AST) and phenotypic detection of ESBL producers". We have also performed a Chi-Square analysis to assess the association between different patient parameters and ESBL production. We have also endeavoured to compare our findings to those reported before.

Any further responses from the reviewers can be found at the end of the article

Background

The intensive care unit (ICU) is a hotspot of nosocomial infections primarily because of the extremely vulnerable population of critically ill patients, usage of invasive procedures such as catheters and ventilators[1,2] and immunosuppressive medication.[3] These infections significantly increase the burden of bacterial associated morbidity, mortality, and healthcare costs. ICU acquired infections (ICU-AI) contribute 20-25% of all nosocomial infections globally.[4] Recent studies have reported high risk of bloodstream infections caused by Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae* among COVID-19 patients admitted in ICU.[5,6]

Antimicrobial resistance (AMR) is a major contributor to the problem of ICU acquired infections. AMR reduces the effectiveness of antibiotics and other antimicrobial drugs in treating these infections. Emergence of AMR leads to a higher risk of treatment failure, longer hospital stays, and increased mortality rates, as well as greater healthcare costs and resource utilization.[7] Drug resistant bacterial pathogens emerge and spread in the ICU environment as a result of acquisition of mutations, and selection of resistant strains, driven mostly by indiscriminate use of antibiotics.[8] Additionally, Gram-negative bacteria have evolved an intrinsic mechanism involving the production of extended spectrum beta lactamases (ESBLs) that breakdown the beta lactam antibiotics.[9] Resistance to antibiotics can be considered multidrug resistance (MDR) when the target organism develops resistance against more than one antimicrobial agent.[10] The outbreak and spread of COVID-19 also contributed to spread of drug resistant bacterial infections in ICU due to the increased number of patients requiring ICU admission. A high prevalence of bacterial pneumonia, 44% (n= 716) among covid 19 patients admitted in ICU has been reported.[11]

Phenotypic resistance to the third generation cephalosporins (cefotaxime, ceftazidime and ceftriaxone) is increasing, posing a significant public health threat.[12,13] Cephalosporins are valuable agents used in the management of a wide range of Gram-negative infections including meningitis, Lyme disease, pseudomonas pneumonia, Gram-negative sepsis, streptococcal endocarditis, melioidosis, penicillinase-producing *Neisseria gonorrhoea*, and Gram-negative osteomyelitis.[14] The use of molecular tools to profile the ESBLs producing Gram-negative bacteria have confirmed the presence of multiple ESBL genes (^{bla}CTX-M, ^{bla}SHV, ^{bla}TEM, ^{bla}OXA) in isolates of *Klebsiella pneumonia, Escherichia coli*, and *Proteus* species, corresponding to high-level resistance to third generation cephalosporins.[15]

The current study sought to profile phenotypic and genetic resistance to cephalosporin in bacteria isolated from ICU patients' samples. Identification of bacterial species and phenotypic susceptibility patterns were conducted using VITEK 2 (bioMérieux). Phenotypically resistant isolates were confirmed by PCR genotyping.

Methods

Study design and study site

This was a cross sectional study carried out between January to June 2021 at Kenyatta National Hospital (KNH). KNH is the largest public referral and teaching hospital in Kenya with a bed capacity of approximately 1800. The hospital serves patients from the capital city with a population of over three million people. The hospital's critical care unit department is composed of the main ICU and several other specialised units including Neurosurgery-CCU, Medical wards-CCU, Surgical ward-CCU, Neonatal-ICU, and the Casualty CCU. In this study, "ICU" to refers to both main ICU and other specialized CCUs.

Ethical approval

This study was approved by the Kenyatta National Hospital (KNH)-University of Nairobi (UON) Ethics and research committee under the study number: P632/11/2020. Additionally, informed consent/assent were sought from participants or kin of the patient in cases of minors or unconscious patients. Written consent was obtained from next of kin for all participants but two. The two cases involved consent obtained from treating ICU physician, where the patients were

incapacitated and their next of kin were unavailable to give consent. This decision was made based on the deferred consent principle backed by the following reasons

1. The research involves minimum harm to the participant

2. The deferment of consent procedure did not adversely affect the rights and welfare of the patient since the genomic testing (PCR) was carried out on the leftover bacterial isolates and not on the human DNA. These bacterial isolates are regarded as residual laboratory samples material

Patient confidentiality and data privacy was ensured by assigning unique study code to each participant. Participant metadata was collected using password protected excel data collection tool.

Study population and sampling

Study participants included all patients admitted to various ICUs in KNH suspected to have bacterial infection during their entire period of admission. Inclusion criteria included having a Gram-negative culture positive specimen. Patients with only Gram-positive cultures were excluded. Sample size was determined using the Cochrane's and Finite population correction for proportions formula.[16]

Antimicrobial Susceptibility Testing (AST) and phenotypic detection of ESBL producers

Sample quality and quantity were reviewed prior to labelling for bacteriology assessment. Degraded samples or those with inadequate volume were excluded. Samples that passed the inclusion criteria were processed for organism identification and antimicrobial susceptibility of culture positive Gram-negative isolates using the Vitek®2 (*Biomérieux, Marcy l'Etoile, France*) with Minimum Inhibitory Concentration (MIC) breakpoints set according to CLSI 2020 guidelines. Prior to loading isolates into the VITEK® 2, bacterial suspensions were prepared by emulsifying the isolates in 0.5% saline and standardizing turbidity to 0.5 McFarland's using a densitometer. The suspension was used for species identification, AST and phenotypic detection of ESBL producing organisms in the VITEK® 2 using Gram-negative cards (GN83). Vitek®2 Advanced Expert System (AES) was used. A commercially acquired Gram negative isolates as positive control was loaded for each run on Vitek. For negative controls we used bacterial suspension media (saline). Antimicrobial susceptibility profiles for Cefotaxime, ceftazidime and ceftriaxone were also recorded. The Minimum Inhibitory Concentrations (MICs) were set according to CLSI 2020 guidelines. For specimens identified phenotypically as ESBL producers, another inoculum was picked from residual specimen and stored in skimmed milk-tryptone-glucose-glycerol broth at -80°C to minimize risk of mutations during batching, awaiting PCR.

PCR Genotyping

Isolates that showed phenotypic resistance to Cefotaxime, ceftazidime and ceftriaxone were selected and used for subsequent PCR genotyping. The Isolate II Genomic DNA kit (Bioline London, UK) was used for total DNA extraction. The kit applies affinity columns to extract genomic DNA. Proteinase K, together with cell lysis buffers containing chaotropic salt ions are used to lyse cells releasing gDNA, which is captured by the affinity resins (silica gel membrane). DNA extraction was followed according to manufacturer's instructions and eluted in a final volume of 40 ul PCR amplification was then performed using MyTaq™ PCR mix (Bioline, London, UK) in a final volume of 20µl, comprising a master-mix, 0.4 µM of each forward and reverse primers and 3 µl of DNA template. Primers specific to ESBL encoding genes (^{bla}TEM, ^{bla}SHV, ^{bla}CTX-M and ^{bla}OXA) were used as described by.[17,18] These ESBL genes were chosen for PCR genotyping since they were most frequently detected based on phenotypic resistance detection.

Briefly, amplicons were analysed by gel electrophoresis run in 1% agarose gel, 1×TAE buffer and SYBR™ Safe (Invitrogen, Carlsbad, CA, USA) and a 1KB ladder at 70 volts for 30 minutes. The amplified products were visualized under Ultraviolet trans-illumination using the UVTEC Gel Documentation Systems (Cleaver Scientific, United Kingdom,) to identify presence of ESBL genes. The commercial *E.coli KEN063* isolate was used as a positive control, while *E.coli 25922* negative control. The primer sequences and thermocycling conditions used in this study are provided in the Supplementary table 1 and Supplementary table 2 in the Data Availability section (DOI: 10.6084/m9.figshare.22369975).

Statistical analysis

Statistical analyses were performed in MS. Excel 2010 and GraphPad Prism (version 8.0.4). Shapiro-Wilk test was used to assess data normality prior to analyses. Descriptive statistics including means and frequencies were used for data summary. Mean comparisons among three or more groups was performed using one-way ANOVA with Tukey's post-hoc. Associations between variables were determined using Chi-Square test. Descriptive data was presented as mean ± SD and data considered statistically significant at p value <0.05.

Table 1. Distribution of bacterial species per sample.

	A. baumanii	*C. freundii*	*E. coloacae*	*E. coli*	*K. pneumoniae*	*K. pneumniae pneumoniae*	*P. aeruginosa*	*S. marcescens*	**Total**
Ascitic tap	1	0	0	0	0	0	0	0	1
Blood	9	0	3	4	3	0	0	0	19
CVC tip	0	0	0	0	0	0	1	0	1
Pus swab	0	0	0	2	2	0	3	2	9
Sputum	1	0	0	0	0	0	0	0	1
T/A	38	3	2	14	23	8	10	1	99
Urine	10	0	1	11	13	0	3	0	38
Total	**59**	**3**	**6**	**31**	**41**	**8**	**17**	**3**	168

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Results

Bacterial abundance per specimen type: Tracheal aspirate specimen had the highest abundance of bacteria

The highest number of bacteria were isolated from tracheal aspirate (TA) (99/168) followed by urine (38/168) and blood (19/168) while ascitic tap, CVC tip and sputum had one isolates each. Table 2 T/A harboured all the isolated tested, with a total of 99 isolates. The distribution of species in T/A showed that *A. baumanii* were the highest in TA (38/99). Urine specimen had the second highest number of species (38/168). Out of the 8 species identified, urine had 5 species, with *K. pneumoniae* being the most frequent, identified 13 times (Table 1).

Isolate distribution

A total of 168-Gram-negative isolates were phenotypically identified from ICU patients' samples. The isolates comprised of 8 Gram-negative bacteria species, with *A. baumanii* being the most abundant (35%) followed by *K. pneumoniae* (24%), and *E. coli*, 18% while the remaining species were present at frequencies ≤10% (Figure 1).

Phenotypic susceptibility and ESB production: Majority of isolates were ESBL producers

Phenotypic susceptibility analysis revealed high level of resistance among the bacterial isolates identified. Overall, 101/168 (60.1%) isolates were ESBL producers while 67/168 (39.9%) were ESBL non-producers. ^{bla}TEM was the most abundant ESBL, occurring in 99/168 followed by shv (88/168), ^{bla}CTX-M (81/168), and ^{bla}OXA (54/168) (Table 2). ^{bla}OXA was produced by most of the organisms, but there were no statistically significant difference when compared to other ESBLs (Table 2).

ESBL production and different parameters

Majority of patients 76% were males and the highest number of bacterial isolates were from patients aged between 21 to 40 years 75/168 and 50 out of the 75 isolates were phenotypically resistant to at one cephalosporin. Conversely, few isolates (3/168) were isolated from patients aged >80 years; all the isolates were phenotypically susceptible to all tested cephalosporins (Table 3).

The susceptibility pattern revealed high level of phenotypic resistance against three cephalosporins (Ceftazidime, Ceftriaxone, and Cefotaxime) (Table 4).

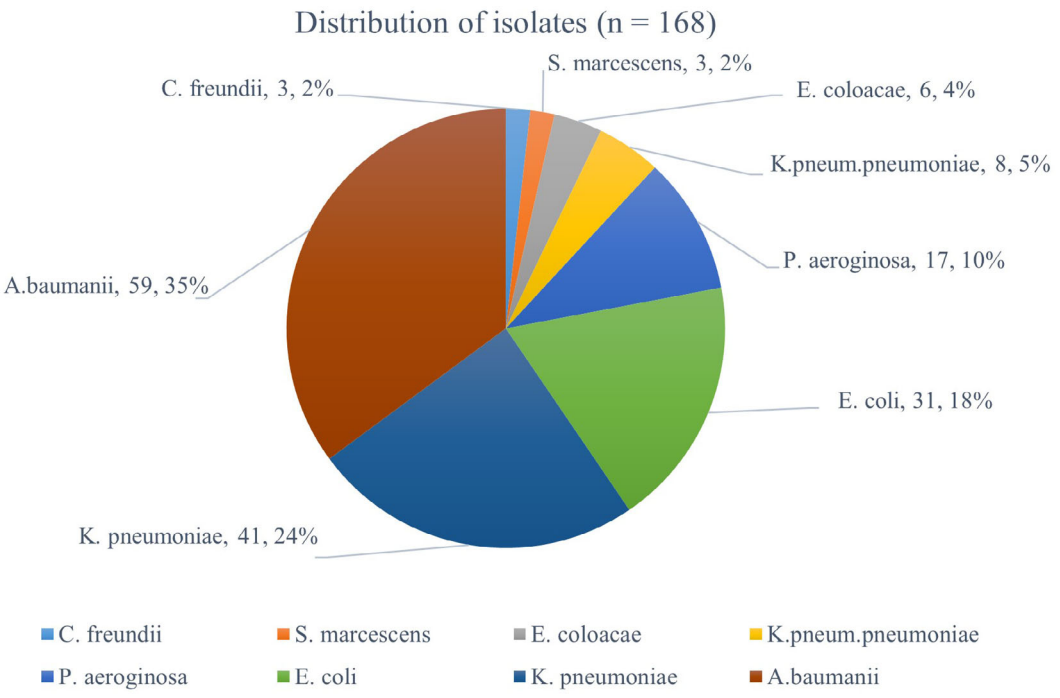

Figure 1. The frequency of Gram-negative bacteria species identified in ICU patient samples.

Table 2. ESBL production by Gram-negative bacterial isolates from ICU patients' specimen.

Species	*bla*TEM		*bla*SHV		*bla*CTXM		*bla*OXA		n	
	ESBL +ve	ESBL -ve	ESBL +ve	ESBL -ve	ESBL +ve	ESBL -ve	ESBL +ve	ESBL -ve	ESBL +ve	ESBL -ve
C. freundii	1	2	1	2	0	3	0	3	1	2
S. marcescens	1	2	1	2	1	2	0	3	1	2
E. coloacae	3	3	3	3	3	3	2	4	3	3
K.pneum. pneumoniae	0	8	0	8	0	8	0	8	0	8
P. aeroginosa	4	13	2	15	2	15	2	15	4	13
E. coli	20	11	20	11	18	13	11	20	20	11
K. pneumoniae	32	9	32	9	31	10	19	22	32	9
A.baumanii	38	2	29	11	26	14	20	20	40	19
Total	**99**	**50**	**88**	**61**	**81**	**68**	**54**	**95**	**101**	**67**

Comparison of various types of ESBL showed that tem was the most abundant ESBL, while oxa was the least. *A. baumani* was the most frequent ESBL producer.

Table 3. Summary of ESBL production and different parameters. P values were obtained by performing chi-square analysis.

		Positive (n=101)	Negative (n=67)	p-value
Age	≤20	10	10	0.159
	21 – 40	50	24	
	41 – 60	27	22	
	61 – 80	14	8	
	>80	0	3	
Gender	Male	82	46	0.083
	Female	19	21	
Specimen type	Ascitic tap	1	0	0.080
	Blood	8	11	
	Pus swab	5	4	
	Sputum	1	0	
	Tracheal aspirate	56	41	
	Urine	30	10	
	CVC* tip	0	1	
Species	*A. baumanii*	40	19	**0.012**
	C. freundii	1	2	
	E. cloacae	3	5	
	E. coli	20	11	
	K. pneumoniae	31	17	
	P. aeruginosa	4	10	
	P. mirabilis	1	0	
	S. marcescens	1	2	
	A. calcoaceticus	0	1	
		Positive (n=101)	**Negative (n=4)**	**p-value**
HIV	YES	2	0	1.000
	NO	100	4	
HYPERTENSION	YES	12	1	0.235
	NO	89	3	
DIABETES	YES	6	2	0.131
	NO	96	2	
COVID-19	YES	7	0	1.000
	NO	95	4	

Table 4. Susceptibility patterns of various bacterial species.

	Ceftazidime			Ceftriaxone			Cefotaxime		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
A. baumannii	8	0	53	3	5	53	6	2	53
C. freundii	2	0	1	2	0	1	2	0	1
E. cloacae	4	0	4	3	0	5	3	0	5
E. coli	7	0	24	3	0	28	3	0	28

Table 4. *Continued*

	Ceftazidime			Ceftriaxone			Cefotaxime		
	S	I	R	S	I	R	S	I	R
K. pneumoniae	7	6	34	4	0	43	4	0	43
Proteus mirabilis	0	0	1	0	0	1	0	0	1
P. aeruginosa	12	1	4	0	0	0	0	1	16
S. marcescens	2	0	1	2	0	1	2	0	1

A. baumanii had the highest resistance to all the three tested antibiotics followed by *K. pneumoniae* and *E. coli* respectively.

Table 5. The isolates that had resistance to all tested cephalosporins and the frequency of resistance genes.

	A. baumanii	E. cloacae	E. coli	K. pneumoniae	P. aeruginosa
	n=41	n=3	n=20	n=33	n=4
^{bla}CTX-M	26 (63.4%)	3 (100.0%)	18 (90.0%)	31 (93.9%)	2 (50.0%)
^{bla}TEM	38 (92.7%)	3 (100.0%)	20 (100.0%)	32 (97.0%)	4 (100.0%)
^{bla}OXA	20 (48.8%)	2 (66.7%)	11 (55.0%)	19 (57.6%)	2 (50.0%)
^{bla}SHV	29 (70.7%)	3 (100.0%)	20 (100.0%)	32 (97.0%)	2 (50.0%)

Molecular analysis detected the ESBL genes in all the bacterial species studied.

Genotypic susceptibility

The 101 isolates that were phenotypically resistant to cephalosporin were subjected to PCR genotyping and 97 (96%) isolates harboured at least one of the four gene tested while four isolates were negative for all the four genes. ^{bla}TEM was the most predominant gene at **96%** (97/101), followed by SHV = 85.6% (86/101), CTX-M = 78.8% (80/101) and OXA= 52.9% (54/101). ^{bla}TEM/^{bla}SHV/^{bla}CTX-M/^{bla}OXA and ^{bla}TEM/^{bla}SHV/^{bla}CTX-M gene combinations were present at **49%** (n=49/101) and **25.7%** (n=26/101) respectively. Other common gene combinations included ^{bla}*TEM/SHV* at 6.7% (n=7/101), ^{bla}*TEM/CTX-M/OXA* at 1.9% (n=2/101), ^{bla}*TEM/SHV/OXA* at 1.9% (n= 2/101), ^{bla}*SHV/CTX-M* at 1.9% (n= 2/101) (Table 5).

Discussion

Bacterial infection in the ICUs represent a major burden and safety concern for patients admitted to the ICU.[19] Patients in ICU are often critically ill and require urgent care. As a result, they are prescribed antimicrobial therapy empirically to manage their condition while waiting for culture result.[4] The World Health Organization (WHO) considers this irrational use of antimicrobial in ICU a major contributor to development of antimicrobial resistance.[20] In light of the rampant use of antibiotics in ICU, this study was conducted to evaluate the level of bacterial colonization in various sample types drawn from ICU patients and the corresponding level of antibiotic resistant Gram-negative bacteria. Additionally, susceptibility to three classes of cephalosporins (Ceftazidime, Ceftriaxone and Cefotaxime) was assessed.

Acinetobacter baumanii, *Klebsiela pneumoniae and E. coli* were the most abundant organisms (35%, 24%, and 18% respectively). The current study corroborates with other studies reporting similar rates of *Acinetobacter* species (30.9%) and *Klebsiella* species (29.7%) followed by *Pseudomonas aeruginosa* (22.9%) in ICU environment.[21] A study that sought to assess the prevalence of ESKAPE, a group of pathogens consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella neumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* showed that *Enterobacter* spp showed that *Klebsiella pneumoniae*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa* were frequently isolated the in ICUs.[22] In yet another study, *Pseudomonas* species was found to be high (29.1%) in ICU setting followed by Acinetobacter (27.5%).[23] The trend in ICU bacterial colonization appears to be dominated by the three main organisms *Acinetobacter species*, *Klebsiella species and Pseudomonas* species, as demonstrated in previous studies[22,23] and corroborated by our study. We also showed that *Acinetobacter baumanii* and *Klebsiela pneumoniae* ICU isolates were resistant to all tested cephalosporins. The resistance to multiple cephalosporins might partially explain the high prevalence of these bacteria in ICUs. Our findings were in agreement with Saxena and colleagues who reported multiple drug resistance in Acinetobacter and Klebsiella.[4]

Organism distribution varied significantly among different specimen types. Tracheal aspirate had the highest isolates (59%) followed by urine (23%) and blood (11%) while ascitic tap, CVC tip and sputum had (0.6%) each.

These findings agreed with previous report of high prevalence (56%) of pulmonary colonization among ICU patients identified by tracheal aspirate culture.[24] Tracheal aspirate culture has been evaluated as a non-invasive method for diagnosis of ventilator-associated pneumonia colonization.[25] The ease of obtaining tracheal aspirate sample and availability of established protocol could explain why more tracheal aspirate samples were obtained and cultured successfully. Urine, blood and pus swabs yielded 23%, 11% and 5% of total organisms respectively. The lower proportion of culture positivity could be influenced by the small number of samples or the culture method used.

Concordant with phenotypic susceptibility findings, we reported high level of genetic resistance in *A. baumanii, K. pneumoniae* and *E. coli. A. baumanii* is an opportunistic nosocomial pathogen that is resistant to most antimicrobial.[26] Resistance to multiple antibiotics could be responsible for the high prevalence in ICU settings. A previous study linked *A. baumanii* to ventilator-associated pneumonia.[27] Carbapenem resistance in *A. baumanii* is mediated by class D β-lactamases belonging to ^{bla}OXA-type. In addition, *A. baumanii* possesses an intrinsic chromosomally encoded oxacillinase ^{bla}*OXA-51*, which may account for the high prevalence of ^{bla}OXA (48.8%) reflecting its ability to resist eradication.[28] A study in hospital wards in neighbouring Uganda investigated carriage of ^{bla}CTX-M, ^{bla}TEM, and ^{bla}SHV genes and showed that 61 (59%) of all isolates carried ESBL-encoding genes, with ^{bla}CTX-M being the heighest (93%, 57/61).[29] Also, a study in Tanzania by Kibwana and colleagues found that ^{bla}CTX-M-15 was the common EBSL gene among admitted febrile children.[30] In study we report 57.6% of *K. pneumoniae* isolates possess ^{bla}OXA gene. Similar findings were recently reported demonstrating the involvement of ^{bla}*OXA* gene in mediating resistance to cephalosporins.[31] The high prevalence cabapenem resistant bacteria in ICU setting has important implications for patient management in these critical care settings especially when patients are critically ill. Nosocomial infections are likely to be common in these settings resulting to high mortality in ICUs. During severe disease outbreaks requiring hospitalization and admission to ICUs, more people are exposed to these infections.

Analysis of ^{bla}CTMX, ^{bla}TEM, and ^{bla}SHV genes revealed a carriage of resistance gene in more than 50% of studied isolates. A study performed previously in an Indonesian hospital reported similar findings.[32] Moreover, molecular surveillance of ESBL in neonates samples from Kenya and Nigeria revealed a high prevalence of ESBL producing bacteria.[33] The high prevalence of ESBL producing bacteria in ICU underscore the need to heighten surveillance of antibiotic resistance to provide the much-needed information to tackle resistance. This study contributes to the understanding of the burden antibiotic resistant bacteria in ICU, which can inform antibiotic use policies to combat resistance. In conclusion, this study has revealed the growing challenge of prevalence of antibiotic resistant bacterial isolated in ICUs. Despite the fragile nature of ICU patients, it continues to be colonized by antibiotic resistant bacterial isolates as we have demonstrated. While there are no definitive measures to eradicate antibiotic resistant bacteria in ICUs, vaccines against these pathogens remain elusive and where available, they are unaffordable. Thus, prudent use of antibiotics in ICU to avoid widespread resistance is recommended. Additionally, the research for development of more potent antibiotics with genetic barrier to resistance should be supported if we are to win the battle against antibiotic resistance.

Data availability

Figshare. Phenotypic and genetic Extended Spectrum Beta Lactamase cephalosporin resistance profiles of bacterial isolates from ICU in Tertiary Level Hospital in Kenya. DOI: https://doi.org/10.6084/m9.figshare.22369975.v2.[34]

This project contains the following data:

Extended Spectrum Beta Lactamase.xlsx: The data contain phenotypic antibiotic susceptibility values for bacterial isolates and genotypic resistance data assessed by detection of ESBL genes is also part of the data.

Raw DATA_VITEK Bacterial identification.xlsx: Bacterial identification readings from VITEK 2.

Supplementary Materials.docx: This file contains the PCR primer sequences and thermocycler conditions.

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References

1. Brusselaers N, Vogelaers D, Blot S: **The rising problem of antimicrobial resistance in the intensive care unit.** *Ann. Intensive Care.* 2011; **1**: 1–7.
Publisher Full Text

2. Wu D, Wu C, Zhang S, *et al.*: **Risk factors of ventilator-associated pneumonia in critically III patients.** *Front. Pharmacol.* 2019; **10**: 482.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Yang X, Yu Y, Xu J, *et al.*: **Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study.** *Lancet Respir. Med.* 2020; **8**: 475–481.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Saxena S, Priyadarshi M, Saxena A, *et al.*: **Antimicrobial consumption and bacterial resistance pattern in patients admitted in ICU at a tertiary care center.** *J. Infect. Public Health.* 2019; **12**(5): 695–699.
PubMed Abstract | Publisher Full Text

5. Saharman YR, Karuniawati A, Severin JA, *et al.*: **Infections and antimicrobial resistance in intensive care units in lower-middle income countries: a scoping review.** *Antimicrob. Resist. Infect. Control.* 2021; **10**: 22.
PubMed Abstract | Publisher Full Text | Free Full Text

6. Giannitsioti E, Louka C, Mamali V, *et al.*: **Bloodstream infections in a COVID-19 Non-ICU department: Microbial epidemiology, resistance profiles and comparative analysis of risk factors and patients' outcome.** *Microorganisms.* 2022; **10**: 1314.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Tran GM, Ho-Le TP, Ha DT, *et al.*: **Patterns of antimicrobial resistance in intensive care unit patients: a study in Vietnam.** *BMC Infect. Dis.* 2017; **17**: 429.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Reygaert WC: **An overview of the antimicrobial resistance mechanisms of bacteria.** *AIMS microbiology.* 2018; **4**: 482–501.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Ferreira CM, Ferreira WA, Almeida NCOS, *et al.*: **Extended-spectrum beta-lactamase-producing bacteria isolated from hematologic patients in Manaus, State of Amazonas, Brazil.** *Braz. J. Microbiol.* 2011; **42**: 1076–1084.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Cantón R, Gijón D, Ruiz-Garbajosa P: **Antimicrobial resistance in ICUs: an update in the light of the COVID-19 pandemic.** *Curr. Opin. Crit. Care.* 2020; **26**: 433–441.
Publisher Full Text

11. Conway M, Andrew, Kohler K, *et al.*: **Co-infection and ICU-acquired infection in COVID-19 ICU patients: a secondary analysis of the UNITE-COVID data set.** *Crit. Care.* 2022; **26**: 236.
PubMed Abstract | Publisher Full Text | Free Full Text

12. Lester R, Musicha P, Van Ginneken N, *et al.*: **Prevalence and outcome of bloodstream infections due to third-generation cephalosporin-resistant Enterobacteriaceae in sub-Saharan Africa: a systematic review.** *J. Antimicrob. Chemother.* 2020; **75**: 492–507.
PubMed Abstract | Publisher Full Text | Free Full Text

13. Nagassar RP, Daniel K, Bridgelal-Nagassar RJ, *et al.*: **Surveillance of phenotypic extended spectrum beta-lactamase resistance in blood isolates at a Hospital in East Trinidad.** *Caribb. Med. J.* 2021.
Publisher Full Text

14. Arumugham VB, Gujarathi R, Cascella M: **Third generation cephalosporins.** 2019.

15. Akenten CW, Khan NA, Mbwana J, *et al.*: **Carriage of ESBL-producing Klebsiella pneumoniae and Escherichia coli among children in rural Ghana: a cross-sectional study.** *Antimicrob. Resist. Infect. Control.* 2023; **12**(1): 60.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Israel GD: **Determining sample size.** 1992.

17. Kiiru J, Kariuki S, Goddeeris BM, *et al.*: **Analysis of β-lactamase phenotypes and carriage of selected β-lactamase genes among Escherichia coli strains obtained from Kenyan patients during an 18-year period.** *BMC Microbiol.* 2012; **12**: 111–155.
Publisher Full Text

18. Saisi H, Makobe C, Kangongo M, *et al.*: **Prevalence of CTXM, SHV, TEM AND OXA Genes among Extended-Spectrum Beta-Lactamase Producing Klebsiella pneumoniae from Mukuru Slum, Kenya.** *Adv. Microbiol.* 2019; **09**: 853–862.
Publisher Full Text

19. Patil RK, Kabera B, Muia CK, *et al.*: **Hospital acquired infections in a private paediatric hospital in Kenya: a retrospective cross-sectional study.** *Pan Afr. Med. J.* 2022; **41**.

20. WHO: **Regional strategy on prevention and containment of antimicrobial resistance, 2010.** 2010.
Reference Source

21. Mehta T, Chauhan B, Rathod S, *et al.*: **Bacterilogical profile and drug resistance pattern of isolates of the patients admitted in medical intensive care unit of a tertiary care hospital in Ahmedabad.** *Med. Sci.* 2015; **4**: 222–225.

22. Bereanu A-S, Bereanu R, Mohor C, *et al.*: **Prevalence of Infections and Antimicrobial Resistance of ESKAPE Group Bacteria Isolated from Patients Admitted to the Intensive Care Unit of a County Emergency Hospital in Romania.** *Antibiotics.* 2024; **13**(5): 400.
PubMed Abstract | Publisher Full Text | Free Full Text | Reference Source

23. Barai L, Fatema K, Haq JA, *et al.*: **Bacterial profile and their antimicrobial resistance pattern in an intensive care unit of a tertiary care hospital of Dhaka.** *Ibrahim Med. Coll. J.* 2010; **4**: 66–69.
Publisher Full Text

24. McCauley LM, Webb BJ, Sorensen J, *et al.*: **Use of Tracheal Aspirate Culture in Newly Intubated Patients with Community-Onset Pneumonia.** *Ann. Am. Thorac. Soc.* 2016; **13**: 376–381.
PubMed Abstract | Publisher Full Text

25. Scholte JB, Van Dessel HA, Linssen CF, *et al.*: **Endotracheal aspirate and bronchoalveolar lavage fluid analysis: interchangeable diagnostic modalities in suspected ventilator-associated pneumonia?.** *J. Clin. Microbiol.* 2014; **52**: 3597–3604.
PubMed Abstract | Publisher Full Text | Free Full Text

26. Sanz-García F, Gil-Gil T, Laborda P, *et al.*: **Coming from the wild: multidrug resistant opportunistic pathogens presenting a primary, not human-linked, environmental habitat.** *Int. J. Mol. Sci.* 2021; **22**: 8080.
PubMed Abstract | Publisher Full Text | Free Full Text

27. Kramer A, Schwebke I, Kampf G: **How long do nosocomial pathogens persist on inanimate surfaces? A systematic review.** *BMC Infect. Dis.* 2006; **6**: 1–8.
Publisher Full Text

28. Roca I, Espinal P, Vila-Farrés X, *et al.*: **The Acinetobacter baumannii oxymoron: commensal hospital dweller turned pan-drug-resistant menace.** *Front. Microbiol.* 2012; **3**: 148.
Publisher Full Text

29. Mayanja R, Muwonge A, Aruhomukama D, *et al.*: **Source-tracking ESBL-producing bacteria at the maternity ward of Mulago hospital, Uganda.** *PLoS One.* 2023; **18**(6): e0286955.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Kibwana UO, Manyahi J, Sandnes HH, *et al.*: **Gastrointestinal colonization of extended-spectrum beta-lactamase-producing bacteria among children below five years of age hospitalized with fever in Dar es Salaam, Tanzania.** *J. Glob. Antimicrob. Resist.* 2022; **30**: 107–114.
PubMed Abstract | Publisher Full Text

31. Al-Sheboul SA, Al-Moghrabi SZ, Shboul Y, *et al.*: **Molecular characterization of carbapenem-resistant Acinetobacter baumannii isolated from intensive care unit patients in Jordanian hospitals.** *Antibiotics.* 2022; **11**: 835.
PubMed Abstract | Publisher Full Text | Free Full Text

32. Candra IK, Yanto F, Suranadi IW, *et al.*: **Characteristic of Extended Spectrum β-Lactamase-Producing Enterobacteriaceae from Fecal Carriage Isolates of Intensive Care Unit Patients at Sanglah Hospital, Bali, Indonesia.** *Open Microbiol. J.* 2021; **15**: 1–6.
Publisher Full Text

33. Edwards T, Williams CT, Olwala M, *et al.*: **Molecular surveillance reveals widespread colonisation by carbapenemase and extended spectrum beta-lactamase producing organisms in neonatal units in Kenya and Nigeria.** *medRxiv.* 2022.2001.2006.22268735. 2022.
Publisher Full Text

34. Magomere E: Phenotypic and genetic Extended Spectrum Beta Lactamase cephalosporin resistance profiles of bacterial isolates from ICU in Tertiary Level Hospital in Kenya. figshare. [Dataset]. 2023.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 22 January 2025

https://doi.org/10.5256/f1000research.174666.r347378

✔ **Tajudeen Oladunni GANIYU**
Fountain University, Osogbo, Osun, Nigeria

I have reviewed the corrected version of the manuscript, and the responses of the authors convince me. I recommend the acceptance of the manuscript in its current form. Thank you for allowing me to review the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Microbiology and Microbial Biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 17 January 2025

https://doi.org/10.5256/f1000research.174666.r347377

✔ **Upendo O Kibwana**
Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

I have reviewed the revised version and all raised comments have been addressed. I recommend approving the current version.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bacteriology, infectious diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 16 October 2024

https://doi.org/10.5256/f1000research.146277.r316326

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Tajudeen Oladunni GANIYU
Fountain University, Osogbo, Osun, Nigeria

The research concept is okay and relevant to the scope of the journal but the manuscript needs major revision. My comments are listed below:

1. The authors should rewrite the abstract to include a clear statement of problem and justification. The conclusion is not emphatic on what needed to be done to address the problem.
2. ESBL are more than four, what are the reasons for limiting the number of gene assessed to just four? State the four genes correctly in the abstract.
3. In the background, third paragraph and third sentence- presence of multiple ESBL genes in isolates of *Klebsiella pneumoniae, Escherichia coli* and *Proteus species*- please list the ESBL genes.

4. In the Results

- how did you differentiate ESBL producers from non ESBL producers?
- tem should be written as TEM, shv should be SHV, ctmx should be CTX-M and oxa should be OXA.

5. In the second paragraph of the results (Phenotypic susceptibility and ESB production- the last sentence, - the differences in the differences in number of ESBL- delete in word in red.

6. Discussion.

- the discussion is poor, despite explaining and interpreting the results, the authors failed to compare their results with those of other authors. Comparing their work with those of other authors will bring out the novelty in their work and also show whether their results are better compared to earlier works.

OTHERS

E. coloacae should be *Enterobacter cloacae*

REFERENCES

- References earlier than 2019 should be updated.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Microbiology and Microbial Biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 10 Nov 2024

Edwin Magomere

Comment 1: The authors should rewrite the abstract to include a clear statement of problem and justification. The conclusion is not emphatic on what needs to be done to address the problem.

Response: We have rewritten the abstract to include the problem statement in the abstract, which now reads as follows:
Problem statement: Bacterial infections in the Intensive Care Units are a threat to the lives of critically ill patients. Their vulnerable immunity predisposes them to developing bacteria-associated sepsis, deteriorating their already fragile health. In the face of increasing antibiotics resistance, the problem of bacterial infection in ICU is worsening.
Justification: Surveillance of bacterial infections in ICUs and drug resistance will help to understand the magnitude of the problem it poses and inform response strategies.

Comment 2: ESBL are more than four, what are the reasons for limiting the number of gene assessed to just four? State the four genes correctly in the abstract.
Response: These four ESBL genes were chosen for PCR genotyping since they were most frequently detected based on phenotypic resistance detection. Also, we restricted our study to 4 genes due to financial constraints

Comment 3: In the background, third paragraph and third sentence- presence of multiple ESBL genes in isolates of *Klebsiella pneumoniae, Escherichia coli* and *Proteus species*- please list the ESBL genes.
Response: We have added the various ESBL genes as recommended and included a reference to it.

Comment 4: How did you differentiate ESBL producers from non ESBL producers?
Response: We used the Phenotypic detection of extended-spectrum β-lactamase production. Specifically, we used the automated VITEK 2 ESBL test (*Biomérieux, Marcy l'Etoile, France*).
Description of how the method work
This method is based on the simultaneous assessment of the antibacterial activity of cefepime, cefotaxime and ceftazidime, measured either alone or in the presence of clavulanate. This test relies on card wells containing 1.0 mg/L of cefepime, or 0.5 mg/L of cefotaxime or ceftazidime, either alone or associated with 10 or 4 mg/L of clavulanate, respectively. After inoculation, cards are introduced into the VITEK 2 machine, and for each antibiotic tested, turbidity is measured at regular intervals. The proportional reduction of growth in wells containing a cephalosporin combined with clavulanate is then compared with that achieved by the cephalosporin alone and is interpreted as ESBL-positive or –negative through a computerized expert system.
We have also edited the subtitle on phenotypic detection to "**Antimicrobial Susceptibility Testing (AST) and phenotypic detection of ESBL producers**".

Comment 5: tem should be written as TEM, shv should be SHV, ctmx should be CTX-M and oxa should be OXA.
Response: We have edited the names of ESBL gene in all instances where they were not written in the right format. Thank you for drawing my attention to this.

Comment 6: In the second paragraph of the results (Phenotypic susceptibility and ESB production- the last sentence, - the differences in the differences in number of ESBL- delete in word in red.
Response: We have deleted the words in red and re-written the sentence to read "*bla*TEM was produced by most of the organisms but there were no statistically significant difference when compared to other ESBLs "

Comments: Discussion.
- the discussion is poor, despite explaining and interpreting the results, the authors failed to compare their results with those of other authors. Comparing their work with those of other authors will bring out the novelty in their work and also show whether their results are better compared to earlier works.
Response: We have edited the discussion to discuss our findings in context of other works in the same area and added more references. Throughout the discussion, we have endeavored to compare results to previous studies.

Competing Interests: There are no competing interests to disclose

Reviewer Report 06 June 2024

https://doi.org/10.5256/f1000research.146277.r217591

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Upendo O Kibwana
Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

The work is relevant but needs major revision to be more understandable and reproducible
See comments below:

1. The authors have to be mindful of how to write scientific names properly (italicized), how to write gram stain " Gram" instead of gram, and correct all the typos. Also how the gens are written, in capital form not small letters.

2. In the abstract the authors are talking about four genes, please indicate clearly which genes are referred to.

3. The authors focus on ESBL genes. is OXA and ESBL gene or beta lactam gene? Please clarify and possibly change the title accordingly.

4. In the abstract the authors are talking about high level of multidrug resistance. Can the authors clarify what MDR is? because this is not indicated anywhere in the manuscript nor is it reflected in the results.

5. It is not clear if this was a laboratory-based cross-sectional study or simply cross cross-sectional study. At some point, it seems the starting point was in the lab with Gram-negative isolates, while in the population section, the authors indicate they started in the ward (ICU) please indicate clearly. If the study started in the lab what was the rationale for obtaining informed consent rather than requesting a waiver for informed consent? If the study started from the ward how did you exclude pats with Gram-positive isolates prior to sample collection?

6. The authors have to explain how they obtained isolates which subjected to identification test on VITEK. Please indicate all quality control measures observed.

7.How many antibiotics were tested during AST? Provide the names and conc.

8. The results section has to be rearranged to provide a better narrative. Start with where the isolates were obtained from, which isolates were isolated from where, then narrow down to the isolated ESBL.

9. Most of the results sections can be improved to describe better what is presented in the tables. e.g. the bacterial abundance section can be improved by adding information on the isolates which were found in one type of specimen only. The ESBL production and different production parameters section can be improved by adding information about other parameters; Would be good to perform a chi-square test to see if the differences between the parameters are significant or coincidental due to numbers.

10. genotypic susceptibility section has to be described more; how many isolates carried one gene only? How many multiple? Which was the most combo of genes? In which isolates were more prominent with multiple genes etc. Also why 2 isolates were negative and not 4? 97 were positive.

11. "The susceptibility pattern revealed high level of phenotypic resistance against three cephalosporins (Ceftazidime, Ceftriaxone, and Cefotaxime)" This sentence seems ectopic where it is placed.

12. In the discussion section the authors should mention the settings of the studies that they make comparisons with e.g. in paragraph two.

13. "Unsurprisingly, we reported *Acinetobacter baumanii* and *Klebsiela pneumoniae* as the most common organisms in ICU and resistant to all tested cephalosporins" This statement appears in the discussion section yet it is not indicated anywhere in your result section. Please discuss the things that are from your results.

14. Table 1 'n' should be 'total'.

15. Table 2 should be table 1. This is based on a comment about rearrangement.

16. "Urine, blood and pus swabs yielded 23%, 11% and 5% of total organisms respectively. The lower proportion of culture positivity could be influenced by the small number of samples as well as the culture method." Please explain the about the culture methods referred to in this statement.

17. Can the authors compare their genotypic results with neighbor countries and give clinical significance.

18. I advice the authors to remove the footnotes on the tables because they are rather captions and not footnotes

19. Table 4 is not clear. What are the numbers provided? Percentages or numbers? Provide numbers and percentages. Table not clear? What about other abx? What is the number of tested isolates for each species?

20. The title for table 5 is not clear. What information does the table present?

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bacteriology, infectious diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Nov 2024

Edwin Magomere

Reviewer 1 comments

Comment 1: The authors have to be mindful of how to write scientific names properly (italicized), how to write gram stain " Gram" instead of gram, and correct all the typos. Also

how the genes are written, in capital form not small letters.
Response: All the typos have been corrected throughout the manuscript and gene names written in the right format
Typos: "gram stain" corrected to "Gram stain",
Italicized all scientific names
Specific gene names have been added to the abstract: *bla*TEM, *bla*CTX-M, *bla*SHV and *bla*OXA.

Comment 2: In the abstract the authors are talking about four genes, please indicate clearly which genes are referred to.
Response: the genes we are referring to include: *bla*TEM, *bla*CTX-M, *bla*SHV and *bla*OXA. These have been added to the abstract.

Comment 3: The authors focus on ESBL genes. is OXA and ESBL gene or beta lactam gene? Please clarify and possibly change the title accordingly.
Response: OXA is an ESBL gene. Other ESBL genes that we studied include: (*bla*CTX-M, *bla* SHV, *bla*TEM). We focused on four ESBL genes.

Response: 4. In the abstract the authors are talking about high level of multidrug resistance. Can the authors clarify what MDR is? because this is not indicated anywhere in the manuscript nor is it reflected in the results.
Response: We have added the definition of MDR: Lines in the background section. The last sentence of conclusion under abstract has been rephrased to read as: "The high prevalence rate of ESBL genes in ICU bacterial isolates shown in this study has a important implication for ICU patient management and general antibiotics use".

Comment 5: It is not clear if this was a laboratory-based cross-sectional study or simply cross cross-sectional study. At some point, it seems the starting point was in the lab with Gram-negative isolates, while in the population section, the authors indicate they started in the ward (ICU) please indicate clearly. If the study started in the lab what was the rationale for obtaining informed consent rather than requesting a waiver for informed consent? If the study started from the ward how did you exclude pats with Gram-positive isolates prior to sample collection?
Response: The starting point of the study was the laboratory. However, we used patient samples that had been collected from ICU patients for clinical purposes. Since the samples were not originally collected for research purposes, KNH-UON Ethical review committee tasked us to obtain consent before samples could be used for research purposes. We also needed patient consent to allow us collect patient information such as co-morbidities.
We used differential media to allow only Gram-negative bacteria to grow. Thus, we used only Gram- negative cultures for our downstream experiments.
Comment 6: The authors have to explain how they obtained isolates which subjected to identification test on VITEK. Please indicate all quality control measures observed.
Response: Samples brought to the laboratory requested by the ICU clinical team were cultured and subjected to Gram stain. Only Gram-negative isolates were analyzed on VITEK. We used commercially acquired Gram negative isolates as positive controls for each run on Vitek. For negative controls we used bacterial suspension media (saline).

Comment 7: How many antibiotics were tested during AST? Provide the names and conc.

Response: Antibiotics tested in this study include Cefotaxime, ceftazidime and ceftriaxone. The list of tested antibiotics has been added to the manuscript. The Minimum Inhibitory Concentrations (MICs) were set according to CLSI 2020 guidelines.
Comment 8: The results section has to be rearranged to provide a better narrative. Start with where the isolates were obtained from, which isolates were isolated from where, then narrow down to the isolated ESBL.
Response: The results have been rearranged to start with various sources of isolates as advised

Comment 9: Most of the results sections can be improved to describe better what is presented in the tables. e.g. the bacterial abundance section can be improved by adding information on the isolates which were found in one type of specimen only. The ESBL production and different production parameters section can be improved by adding information about other parameters; Would be good to perform a chi-square test to see if the differences between the parameters are significant or coincidental due to numbers.
Response 1: we have added more information about species identified in specimen types with the highest number of species including the following:
T/A harboured all the isolated tested, with a total of 99 isolates. The distribution of species in T/A showed that *A. baumanii* were the highest in TA (38/99). Urine specimen had the second highest number of species (38/168). Out of the 8 species identified, urine had 5 species, with *K. pneumoniae* being the most frequent, identified 13 times.
Response 2: Table 3 has been replaced to show the outcome of Chi-Square analysis to show differences in various parameters

Comment 10: genotypic susceptibility section has to be described more; how many isolates carried one gene only? How many multiple? Which was the most combo of genes? In which isolates were more prominent with multiple genes etc. Also why 2 isolates were negative and not 4? 97 were positive.

Response: Added information on
. ***bla***TEM was the most predominant gene at **96%** (97/101), followed by SHV = 85.6% (86/101), CTX-M = 78.8% (80/101) and OXA= 52.9% (54/101). *bla*TEM/*bla*SHV/*bla*CTX-M/*bla*OXA and *bla* TEM/*bla*SHV/*bla*CTX-M gene combinations were present at **49%** (n=49/101) and **25.7%** (n=26/101) respectively. Other common gene combinations included *bla*TEM/SHV at 6.7% (n=7/101), *bla*TEM/CTX-M/OXA at 1.9% (n=2/101), *bla*TEM/SHV/OXA at 1.9% (n= 2/101), *bla* SHV/CTX-M at 1.9% (n= 2/101).
Comment 11: "The susceptibility pattern revealed high level of phenotypic resistance against three cephalosporins (Ceftazidime, Ceftriaxone, and Cefotaxime)" This sentence seems ectopic where it is placed.
Response: This sentence has been deleted as advised

Comment 12: In the discussion section the authors should mention the settings of the studies that they make comparisons with e.g. in paragraph two.
Response: The settings of previous studies have now been mentioned in the discussion section

Comment 13: "Unsurprisingly, we reported *Acinetobacter baumanii* and *Klebsiela*

pneumoniae as the most common organisms in ICU and resistant to all tested cephalosporins" This statement appears in the discussion section yet it is not indicated anywhere in your result section. Please discuss the things that are from your results.

Response: This sentence has been deleted

Comment 14: Table 1 'n' should be 'total'.
Response: "n" has been edited to "Total"

Comment 15: Table 2 should be table 1. This is based on a comment about rearrangement.
Response: Tables have been rearranged accordingly

Comment 16: "Urine, blood and pus swabs yielded 23%, 11% and 5% of total organisms respectively. The lower proportion of culture positivity could be influenced by the small number of samples as well as the culture method." Please explain the about the culture methods referred to in this statement.

Comment 17: Can the authors compare their genotypic results with neighbor countries and give clinical significance.
Response: Results have been compared to those obtained from neighboring countries (Tanzania and Uganda) and Clinical significance highlighted**.**

Comment 18: I advice the authors to remove the footnotes on the tables because they are rather captions and not footnotes
Response: Footnotes have been removed from all tables as advised

Comment 19: Table 4 is not clear. What are the numbers provided? Percentages or numbers? Provide numbers and percentages. Table not clear? What about other abx? What is the number of tested isolates for each species?
Response: Number provided in the table is number of isolates in the categories of **S**-susceptible, **I**-intermediate and **R**-resistant. This has been clarified in the revised manuscript.
Comment 20: The title for table 5 is not clear. What information does the table present?
Response: The title has been edited to read as "The isolates that had resistance to all tested cephalosporins and the frequency of resistance genes".

Competing Interests: No competing interests to declear