EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Prevalence and Determinants of Carbapenem Resistance Among ESBL-Producing *Enterobacteriaceae* in Biological Specimens at Kilimanjaro Christian Medical Centre, Moshi, Tanzania

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ABSTRACT

Background: Carbapenem resistance represents a critical global health threat, as it undermines the efficacy of last resort antibiotics used to manage infections caused by multidrug-resistant, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*. The burden is disproportionately higher in low- and middle-income countries, particularly in Africa, due to economic constraints and limited healthcare infrastructure. In sub-Saharan Africa, data on the prevalence and distribution of carbapenem resistance remain limited; however, existing studies highlight its growing emergence among the ESBL-producing *Enterobacteriaceae* in the region.

Objectives: The aim of this study was to determine the prevalence and determinants of carbapenem resistance among ESBL-producing *Enterobacteriaceae* isolated from biological specimens at Kilimanjaro Christian Medical Centre.

Methodology: A cross-sectional laboratory-based study was conducted from April to June 2023 at KCMC University, analysing 166 Enterobacteriaceae isolates from urine, pus, and blood. The production of ESBL was confirmed by phenotypic carbapenemase screening via the modified carbapenem inactivation method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Socio-demographic and clinical data were retrieved using data extraction sheets. Statistical analysis was performed using SPSS version 27, and a *P* value of <.05 was considered statistically significant.

Results: A total of 166 ESBL-producing Enterobacteriaceae were tested for carbapenemase production, 36 (21.7%) revealed to be resistant to carbapenem. The distribution of carbapenemase production E. coli were most prevalent at 23 (13.8%) followed by Citrobacter freundii at 7 (4.2%). Multivariable analysis revealed history of hospital admission within the past three months (AOR 6.28, 95% confidence interval [CI], 1.79 to 22.04; *P*=.004), the use of indwelling devices during the study period (*P*=.038), and the presence of comorbid conditions (*P*=.014) as independent predictors of carbapenem resistance.

Conclusion: The substantial prevalence observed underscores the urgent need to strengthen antimicrobial stewardship, enhance infection prevention strategies, and improve routine surveillance systems in resource-limited healthcare settings.

BACKGROUND

Antimicrobial resistance (AMR) is a major global threat to health, food security, and development.[1] As microorganisms evolve, existing treatments become less effective, making infections harder to manage. This growing resistance leads to treatment failures and increased health burdens, particularly in regions with limited access to alternative therapeutic options.[2] Globally, it is estimated that more than 700,000 people die annually because of AMR and the trend is predicted to reach 10 million deaths per annum in 2050, if no appropriate measures are taken to halt its progress.[3] In the USA, more than 2.8 million people were infected by severe antibiotic resistant infections, and more than 35,000 die from these infections every year. It is estimated that by 2030, if it is left unaddressed, the world will incur an annual cost of about US$ 1 trillion.[4]

Similarly, in Africa, emergence of AMR is a public health problem and a more critical problem, mainly due to unstable regulations in antibiotic prescription as well as unstable and inconsistent surveillance system on antimicrobial use, hence raises more concern in developing countries.[5] For instance, in 2012, approximately 19,400 neonates in Nigeria died from severe antibiotic-resistant pathogens.[4] In Tanzania, the research about carbapenem resistance has previously been done showing resistance to meropenem from clinical isolates, but particularly *Pseudomonas* species,[4] and a study on extended-

spectrum beta-lactamase (ESBL) producers, where carbapenem-resistant *Enterobacteriaceae* were isolated from environmental samples along Msimbazi River Basin Ecosystem in Dar es salaam.[6]

Carbapenems are considered last-resort antibiotics for the treatment of severe infections caused by multidrug-resistant Gram-negative bacteria, particularly those producing ESBLs. However, the increasing emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) has become a major global public health concern. Carbapenem resistance significantly limits treatment options, leading to increased morbidity, mortality, prolonged hospital stays, and higher healthcare costs.[7,8] The spread of carbapenem-resistant pathogens is often driven by the production of carbapenemase enzymes, such as *Klebsiella pneumoniae* Carbapenemase (KPC), New Delhi Metallo-β-lactamase (NDM), …(VIM), and Oxacillinase-48 (OXA-48), which can be easily transmitted between bacteria through mobile genetic elements.[9] The consequences of CRE on acceleration to AMR are highlighted including prolonged hospital stay due to slow recovery process, higher medical expenses, morbidity, mortality and reduced quality of life at both individual and community levels by affecting productivity of patients and caretakers.[10] Different strategies have been established to fight against AMR as WHO introduced the global One Health Programme, and the World Antimicrobial Awareness Week, providing support to countries to implement their national action plan, developing guidance, antimicrobial surveillance stewardship programs, gathering data and evidence to shape polices and drive actions which are implemented in most African countries including Tanzania.[11]

Despite the global concern of CRE, data on the prevalence, risk factors, and resistance patterns remain limited in many low- and middle-income countries, including Tanzania.[12,13] Local surveillance is essential to guide appropriate empirical therapy, inform infection prevention strategies, and support antimicrobial stewardship programs in hospitals such as Kilimanjaro Christian Medical Centre (KCMC). This study, therefore, aimed to address the limited prevalence data on CRE within the context of AMR. It examines the prevalence patterns and determinants of carbapenem resistance in extended-spectrum beta-lactamase-producing *Enterobacteriaceae* from biological specimens at Kilimanjaro Christian Medical Centre. The findings will highlight the needs to advance healthcare practices by informing strategies that curb the spread of resistant pathogens, enhance infection prevention, and improve patient outcomes in clinical settings.

METHODOLOGY

Study Design and Study Area

This was a cross-sectional laboratory-based study conducted from April 2023 to June 2023. The study was carried out at KCMC, Department of Clinical Laboratory, located in Moshi, Kilimanjaro Region, northeastern Tanzania. KCMC is a tertiary referral and zonal consultant hospital serving the northern zone of Tanzania, including Kilimanjaro, Arusha, Tanga, and Manyara regions. The hospital has a bed capacity of approximately 630 and provides a wide range of specialised healthcare services, including advanced diagnostic laboratory services. It is located approximately 70 km from the city of Arusha on the slopes of Mount Kilimanjaro (M8JG+2X7 on Google Maps).

Study Population and Eligibility Criteria

The study population were all the ESBL-positive isolates from urine, blood and pus of patients' samples with complete clinical and demographic information available at the KCMC Microbiology Department. The ESBL-positive isolates were excluded if the corresponding patient samples had incomplete or missing clinical and demographic information. Additionally, isolates that were contaminated, improperly stored, or had insufficient sample volume for confirmatory testing were not included.

Sample Size and Sampling Procedure

The sample size of this study was determined using Cochran's formula for cross-sectional studies. The calculation was based on the proportion 13.4% of a study done in Zanzibar.[14]such as E. coli, has been increasing worldwide, which causes treatment failure for urinary tract infections. Therefore, this study aimed to determine the prevalence and risk factors for the production of ESBL in E. coli from patients with urinary tract infections (UTI

Where:
s=required sample size

Z=standard normal deviate corresponding to a 95% confidence level (1.96)

p=estimated proportion of the population with the characteristic of interest

e=margin of error (0.05).

Thus,

$s=z^2(p(1-p)/ e^2$

$S=\frac{1.96^2 \text{ X } 0.134(1-0.134)}{0.05^2}$

S=178

To account for a potential 10% non-response or unusable samples, an additional 18 isolates were included, bringing the final targeted sample size to 196. However, during the study period, only 166 eligible ESBL-positive isolates with complete clinical and demographic data were available and therefore included in the final analysis.

A purposive sampling technique was employed to include all eligible ESBL-positive isolates obtained from urine, blood, and pus specimens that met the inclusion criteria within the study period.

Data Collection and Study Procedure

Data were extracted from the laboratory data sheet and electronic medical record system (eHMS), including age, sex, ward, prior exposure to healthcare facility, type of sample, laboratory number of the sample, comorbidities of patients and history of admission.

The confirmed ESBL-producing *Enterobacteriaceae* was sub cultured on Blood Agar using sterile cotton swab in ambient air at 35°C overnight to obtain fresh bacterial growth. Modified Carbapenem Inactivation Methods (mCIM) was used to identify CRE. The appropriate

amount of colony from blood agar was picked using sterile cotton swab to normal saline tube for emulsification and the vortex mixer was used to make suspensions of colonies. Then 10µg Meropenem disks were added to the suspension made and incubated at 35°C for 4 hours (socked meropenem disks). A 0.5 McFarland suspension control was used to make the suspensions of *E. coli* ATCC 25922, the standard organism in normal saline and the suspension was inoculated on a 100mm Muller Hinton agar (MHA) plate using the lawn culture method at a time and placed socked meropenem and fresh meropenem disks for control and incubated MHA plates at 35°C to 37°C overnights in aerobic condition. *K. pneumoniae* ATCC 700603 was used for quality control. The results were reported as positive or negative based on recommendations of CLSI.

Data Quality Assurance

Quality control was performed on all biochemical identification tests and all media by using ATCC control strains. For performance control, *E. coli* ATCC 25922 (Ref. R4601971. Thermo Scientific, Lenexa, KS 66215 USA), *Pseudomonas aeruginosa* ATCC 27065 (Ref. R4607892. Thermo scientific Lenexa, KS 66215 USA) and *S. aureus* ATCC 25923 (Ref. R4609022. Thermo Scientific, Lenexa, KS 66215 USA) strains were used as reference strains. These control Bacteria were inoculated on MCA for lactose and non-lactose fermenters respectively and *S. aureus* onto blood agar. Also, these strains were used in different identification tests such as; catalase test, coagulase test and oxidase test. For sterility control, the media were incubated at 37°C, incubator for 18 to 24 hours to see if the media were contaminated or not.

Statistical Analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) software, version 27 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used for patients' socio-demographic characteristics. Logistic regression analysis was conducted to determine factors associated with carbapenem resistance among ESBL-producing *Enterobacteriaceae*. Risk factors were assessed using odds ratios (ORs) and 95% confidence intervals (CIs). A *P* value of less than .05 was considered statistically significant to identify independent predictors of carbapenem resistance.

Ethical Considerations

The study was approved by KCMC University ethical committee with certificate number UG 02/2023. Permission to conduct the study was granted from Kilimanjaro Christian Medical Centre administration via the Head of the Clinical Laboratory department. Confidentiality of the participants' information was strictly maintained throughout the study. Data were anonymised by assigning unique identification codes instead of personal identifiers, and all collected information was accessible only to the research team. The data were securely stored and used solely for research purposes.

RESULTS

Socio-demographic Characteristics of Patients

A total of 166 clinical isolates were obtained, of which 116 (69.9%) were from male patients. The age of the patients ranged from 3 days to 95 years. The majority of the patients were aged more than 40 years (53%). Patients' samples were collected from the four main wards: medical, surgical, pediatric and urology, Table 1.

TABLE 1: Social Demographic Characteristics of Patients (N=166)

Variables	Frequency (n)	Percentage (%)
Age in years		
<20	32	19.3
20-40	46	27.7
>40	88	53
Sex		
Males	116	69.9
Females	50	30.1
Admission departments		
Medical wards	82	49.4
Urology wards	48	28.9
Pediatric wards	17	10.2
Surgical wards	19	11.4

Characteristics of Enterobacteriaceae Isolated From Clinical Samples

A total of 166 *Enterobacteriaceae* isolates were phenotypically confirmed to be ESBL producers by using double disc diffusion method. Among the *Enterobacteriaceae* species tested, 97 (58.4%) were from urine samples, 49 (29.5%) from pus and 20 (12.1%) from blood. The most frequent isolates (n=104, 62.7%) were *E. coli* followed by *K. pneumoniae* (n=25, 15.1%), Table 2.

TABLE 2: Characteristics of *Enterobacteriaceae* isolated from clinical sample (N=166)

Variables	Frequency (n)	Percentage (%)
Type of sample		
Urine	97	58.4
Pus	49	29.5
Blood	20	12.1
Type of bacteria isolated		
E. coli	104	62.7
K. pneumoniae	25	15.1
K. oxytoca	5	3
Proteus mirabilis	1	0.6
Proteus vulgaris	1	0.6
Serratia marcescens	2	1.2
Enterobacter aerogenes	4	2.4
Enterobacter cloacae	2	1.2
Citrobacter freundii	18	10.8
Providencia rettgeri	1	0.6
Morganella morganii	3	1.8

Prevalence of Carbapenem Resistance in ESBL-Producing *Enterobacteriaceae*

A total of 166 *Enterobacteriaceae* ESBL producers were tested for carbapenemase production, whereby

36 (21.7%) revealed to be resistant to carbapenem (Meropenem). The carbapenemase production was most prevalent in *E. coli* (n=23, 13.8%), followed by *Citrobacter freundii* (n=7, 4.2%), Table 3.

Determinants of Carbapenem Resistance in ESBL-Producing *Enterobacteriaceae*

In univariate logistic regression analysis, factors for carbapenem resistance that showed statistical significance were comorbid conditions (Crude Odds Ratio [COR] 3.393; 95% Confidence Interval [CI], 1.481 to 7.777; *P*=.003), history of admission in the last three-months (COR 2.810; 95% CI, 1.049 to 7.523; *P*=.034) and the use of any indwelling devices such as urinary catheter, ventilator and nasogastric tube during study period (COR 2.27; 95% CI, 0.99 to 5.209; *P*=.049). Factors like prior exposure to any healthcare facility in last three-months, age category and sex of patients were not statistically significant (*P*>.05), Table 4.

After controlling for confounders using multivariable logistic regression analysis, the history of hospital admission within the past three months (AOR 6.28; 95% CI, 1.79 to 22.04; *P*=.004), the use of indwelling devices during the study period (*P*=.038), and the presence of comorbid conditions (*P*=.014) were identified to be a risk factors for carbapenem resistance among ESBL-producing *Enterobacteriaceae*. (Table 4).

TABLE 3: Distribution of *Enterobacteriaceae* Isolates with Carbapenem Status (N=166)

Bacteria type	Frequency n (%)	carbapenem status	
		Positive n (%)	Negative n (%)
Escherichia coli	104(62.7)	23(22.3)	81(77.9)
Klebsiella pneumoniae	25(15.1)	1(4)	24(96)
Klebsiella oxytoca	5(3.0)	1(20.0)	4(80.0)
Citrobacter freundii	18(10.8)	7(38.9)	11(61.1)
Serratia marcescens	2(1.2)	0(0.0)	2(100.0)
Morganella morganii	3(1.8)	1(33.3)	2(66.7)
Proteus mirabilis	1(0.6)	0(0.0)	1(100.0)
Enterobacter cloacae	2(1.2)	0(0.0)	2(100.0)
Enterobacter aerogenes	4(2.4)	1(25.0)	3(75.0)
Providencia rettgeri	1(0.6)	0(0.0)	1(100.0)
Proteus vulgaris	1(0.6)	1(100)	0(0.0)

TABLE 4: Determinants of Carbapenem Resistance in Extended-Spectrum Beta-Lactamase (ESBL)-Producing Enterobacteriaceae (N=166)

Variables	Total Tested(n)	CRE n (%)	Univariate Analysis		Multivariable Analysis	
			COR (95%CI)	*P-Value*	AOR (95%CI)	*P-Value*
Age category						
<20	32	5(15.6)	1			
20-40	25	2(8)	0.47(0.083-2.653)	.392		
>40	109	29(26.6)	1.958(0.689-5.563)	.208		
Sex						
Male	116	26(22.4)	0.865(0.382-1.963)	.729		
Female	50	10(20)	1			
History of admission within last three months						
No	20	8(40.0)	1			
Yes	146	28(19.2)	2.810(1.049-7.523)	.034	6.28(1.79-22.04)	.004
Having any comorbid condition						
No	78	9(11.5)	1			
Yes	88	27(30.7)	3.393(1.481-7.777)	.003	3.036(1.254-7.352)	.014
Using any indwelling device during study period						
No	65	9(13.8)	1			
Yes	101	27(26.7)	2.27(0.990-5.209)	.049	3.092(1.064-8.985)	.038
Prior exposure to healthcare facility in last three month						
No	21	4(19.0)	1			
Yes	145	32(22.1)	1.204(0.378-3.831)	.754		

Abbreviations, COR =crude odds ratio, AOR=Adjusted odds ratio, CI=confidence interval.
CRE=carbapenem-resistant *Enterobacteriaceae*

DISCUSSION

The current study demonstrated a carbapenem-resistant *Enterobacteriaceae* (CRE) prevalence of 21.7% among ESBL-producing Enterobacteriaceae isolates, indicating a substantial burden of carbapenem resistance in the study setting. This prevalence is higher compared to the prevalence of the study conducted in Northwest Ethiopia, 6.7%,[15] in Korea, 2.6%,[16] in Mumbai-India, 12.26%,[17] and in Zhejiang-China, 15.8%.[18] The variation in prevalence across regions may reflect differences in antimicrobial prescribing practices, infection prevention and control (IPC) measures, diagnostic capacity, and surveillance systems. In low- and middle-income countries, limited laboratory infrastructure and weak antimicrobial stewardship programs may contribute to delayed detection and uncontrolled spread of resistant strains.[1,2,11]

The present study demonstrated a strong association between the presence of comorbid conditions, prior hospital admission, and the use of indwelling medical devices during hospitalisation and the occurrence of resistant isolates ($P<.05$). These findings suggest that increased healthcare exposure and underlying patient vulnerability play a critical role in the acquisition of multidrug-resistant organisms.[19,20] Recent studies have also reported similar risk factors for multidrug-resistant infections. A case-control study in Chongqing, China, found that recent hospitalisation, prior urological procedures, and indwelling catheter use were significantly associated with ESBL-producing *Escherichia coli* infections ($P<.05$).[19] Likewise, a study conducted in tertiary hospitals in Zanzibar, Tanzania, reported that previous hospitalisation, longer hospital stays, and prior antibiotic therapy were independently associated with ESBL-producing *E. coli* urinary tract infections.[20] In addition, research from the United States highlighted the role of indwelling devices in CRE infections. Patients with multiple devices had an increased risk of adverse outcomes, and prior hospitalisation within 90 days was a significant predictor of treatment failure in CRE cases.[21,22] Furthermore, a systematic review from West Africa emphasised that healthcare exposure, prolonged hospital stay, and limited infection control infrastructure contribute to the high burden of carbapenem resistance in hospital settings.[23]

Moreover, studies in Ethiopia,[24] in China,[25] and in the USA,[26] show the statistically significant association between multidrug resistance and long hospital stays, exposure to drugs as well as irrational use of the drugs. Despite this association, current study shows that prior exposure to any health care facility in last three months and sex of participants had no statistical significance. This align with a pediatric crosssectional study done in Aga Khan University Hospital in Pakistan found that none of the examined risk factors, including prior healthcare exposures, were significantly associated with multidrug resistant organisms (MDRO) carriage upon admission.[27] Similarly, a tailored MDRO screening study in an intensive care setting reported that prior admission and sex showed no statistically significant associations with MDRO colonization, despite confirming antibiotic exposure as a risk factor.[28] This suggests that certain demographic or recent exposure factors may not consistently predict multidrug-resistant infections, highlighting the need to focus preventive efforts on well-established risk factors such as prolonged hospital stay and antibiotic stewardship.

Therefore, the current findings underscore the urgent need to strengthen antimicrobial stewardship programs, implement routine surveillance for carbapenem resistance, enhance infection prevention and control measures, and promote rational antibiotic use in healthcare facilities. Policymakers and healthcare institutions should prioritise coordinated One Health approaches to effectively mitigate the growing burden of antimicrobial resistance.

Strengths and Limitations of the study

This study provides important insights into carbapenem resistance among ESBL-producing *Enterobacteriaceae* in a hospital setting, highlighting epidemiological patterns and associated risk factors in a resource-limited environment. The use of confirmed phenotypic methods for ESBL and carbapenemase detection ensured reliable microbiological data, while the inclusion of complete clinical and demographic information allowed meaningful analysis of contributory determinant. However, the relatively small sample size restricted the depth of multivariable analysis and hindered the investigation of true causation in a bivariable context. Additionally, selection bias was introduced by focusing solely on individuals in a hospital setting.

CONCLUSIONS

The study demonstrates a notable presence of carbapenem resistance among ESBL-producing *Enterobacteriaceae*, with *Escherichia coli* and *Citrobacter freundii* being the most prevalent. These findings reflect the rising trend of resistance over time and underscore the critical threat posed by multidrug-resistant bacteria. Significant risk factors identified included recent hospitalizations, existing comorbidities, and the use of medical devices during the study period. The results highlight the urgent need for strengthened infection prevention and control measures, as well as judicious use of antibiotics, to mitigate the spread of resistant strains. Future research employing a retrospective case-control design would be more suitable for establishing causal relationships regarding carbapenem-resistant *Enterobacteriaceae*. Additionally, incorporating advanced molecular techniques, such as polymerase chain reaction (PCR), would enhance detection sensitivity and specificity, providing valuable insights into the genotypic characteristics and resistance mechanisms of the bacteria.

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Peer Reviewed

Acknowledgments: We extend our gratitude to the patients whose samples were included in this study.

Competing Interests: The authors declare no competing interests.

Funding: The study did not receive any funding

Received: 30 June 2025; **Accepted:** 25 March 2026

Cite this article as Issangya TE, Mhina L, Mwampamba T, Kamwela E,, Benjamin A, Juma M, Shoo G, Matowo V, Vuai SM, Kajeguka CD. Prevalence and Determinants of Carbapenem Resistance Among ESBL-Producing Enterobacteriaceae in Biological Specimens at Kilimanjaro Christian Medical Centre, Moshi, Tanzania. *East Afr Science J*. 2026: 8(1): 20-26. https://doi.org/10.24248/easci.v8i1.131