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Phenotypic and Molecular Characterization of Extended-Spectrum β -Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from Communities in South Western Uganda

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Abstract

Background: *Escherichia coli* and *Klebsiella pneumoniae* are common Extended-spectrum β -Lactamase (ESBL) producing bacteria that can potentially cause substantial challenges in both human and veterinary medicine. This study aimed to characterize the phenotypic and genotypic ESBL production in *E. coli* and *K. pneumoniae* isolates from community samples obtained from South Western Uganda.

Methods: We conducted a cross-sectional study using laboratory methods among stored *E. coli* and *K. pneumoniae* isolates. Phenotypic detection of

ESBL production was performed using double disk synergy test and the combination disk test, using the Kirby-Bauer disk diffusion method. DNA Polymerase Chain Reaction (PCR) was performed using a conventional PCR thermocycler to detect the ESBL genes. Descriptive statistics were used to summarize the resistance patterns between *E. coli* and *K. pneumoniae*.

Results: Among the 136 isolates retrieved 96(70.59%) were *E. coli*. The prevalence of ESBL production among isolates was 37.50% (95% CI=29.58-46.52). Among *E. coli*, majority carried blaCTX-M (61.46%) and blaTEM (56.25%) genes. Among *K. pneumoniae* isolates, there was a uniform distribution of the multiplex genes for blaCTX-M (37.5%), blaOXA (32.5%), blaTEM (30%) and SHV (30%).

Conclusion: The blaCTX-M and blaTEM genes were identified as the predominant ESBL genes especially among *E. coli* isolates which aligns with global trends.

Key words

Phenotypic, Molecular characterization, ESBL production, Enterobacterales, Mbarara Uganda

Introduction

Extended-spectrum beta-lactamase (ESBL) production among *E. coli* and *K. pneumoniae* have become a significant public health concern¹. ESBL genes can confer resistance to multiple antibiotics, particularly the beta-lactam antibiotics². The emergence and spread of ESBL producing bacteria in communities poses substantial therapeutic challenges for both human and veterinary medicine, as it diminishes the efficacy of available treatments and raises the possibility of treatment failure, promoting further spread of resistant strains^{3,4}.

While ESBL-producing enterobacterales have been extensively studied in hospital settings, there is growing concern about their emergence and

dissemination in community environments⁵. Community-associated ESBL infections pose a unique public health challenge due to their potential for silent transmission through contaminated food, water, and direct human contact⁶. Transmission within communities and exchanging ESBL-producing characteristics among bacterial strains is common⁷. This raises concerns about the potential transmission of drug-resistant bacteria in the communities and increases the risk of healthcare-associated and community-acquired infections (CAI)³.

In low and middle-income settings such as Uganda, factors including widespread empirical antibiotic use, limited regulation of antimicrobial access, and close interaction between humans, livestock, and the environment may facilitate the spread of resistant organisms beyond healthcare facilities⁶. In Uganda, the occurrence of ESBL-producing enterobacterales has been increasingly reported in both clinical and community settings, with studies demonstrating a substantial prevalence among *Escherichia coli* and *Klebsiella pneumoniae* isolates⁸. For example, a study conducted at Mulago National Referral Hospital reported a high prevalence of ESBL-producing enterobacterales, particularly among *E. coli* and *K. pneumoniae*, highlighting the growing burden of antimicrobial resistance in the country⁹. However, most studies in Uganda have focused on hospital-based isolates, with limited data on the molecular characteristics of ESBL-producing organisms circulating in community settings^{10,11}.

Understanding the phenotypic and genotypic characterization of ESBL in *E. coli* and *K. pneumoniae* isolates from communities is crucial for several reasons. Primarily, it provides insights into the prevalence and distributions of ESBL-producing bacteria in different populations, aiding in the identification of risk factors and probable transmission sources¹². Characterizing the specific ESBL genes also allows for the tracking of resistant strains and understanding their mechanisms of resistance¹³.

This study therefore aimed to characterize the phenotypic and genotypic profiles of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in southwest Uganda, in order to better understand their distribution and inform targeted antimicrobial resistance control strategies.

Materials and Methods

Study Design and location

The study design was a laboratory based descriptive cross-sectional study. Quantitative data was collected to determine the phenotypic and genotypic characterization of ESBL producing *E. coli* and *K. pneumoniae*. the study was conducted at Mbarara University of Science and Technology (MUST) Microbiology laboratory in the MUST- Mbarara Regional Referral Hospital complex located in Mbarara city. The microbiology laboratory at MUST offers complementary laboratory services to the referral hospital and functions as research and training laboratory withing the university.

Study population

The study population comprised of stored isolates at Mbarara University Microbiology laboratory. The isolates were obtained from human, livestock and environmental samples collected in a previous One Health multi-center study conducted in 2019¹¹. The samples were collected from farmers in different households in communities of Bwizibwera and Rubaya sub counties. The collected samples were then cultured overnight on Cysteine Lactose and Electrolyte Deficiency (CLED) agar and Blood Agar (BA). Colonies indicative of growth were identified by gram staining and further biochemical testing. Pure colonies were appropriately labelled and stored in Tryptic soy broth in an isolate bank in the microbiology laboratory.

Eligibility criteria

All *E. coli* and *K. pneumoniae* isolates previously obtained from human, livestock, and environmental samples were considered eligible. Isolates were

excluded if they demonstrated poor growth on subculture, had an inconclusive species level identification or lacked adequate trackable laboratory data.

Data collection methods

Isolate retrieval from the biobank

All stored *E. coli* and *K. pneumoniae* isolates from the previous One health multi-center study were retrieved between 1st November 2023 to 31st March 2024 from the biobank at Mbarara University of Science and Technology Microbiology laboratory for analysis in this study. Samples were then freeze thawed and sub cultured on MacConkey agar with crystal violet for recovery. The plates were incubated at 37°C for 24 hours. Strains were characterized using standard microbiological techniques including biochemical tests and colony morphology. Isolates were tested for indole production, motility, methyl red, Voges Proskauer, citrate and urease production.

Phenotypic detection for ESBL among *E. coli* and *K. pneumoniae*

Phenotypic detection of ESBL production was initially performed using the double-disk synergy test (DDST) on Mueller-Hinton (MH) agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines¹⁴. Colony suspensions prepared with sterile saline and adjusted to a turbidity equivalent to 0.5 McFarland standard were inoculated onto MH agar. An amoxicillin-clavulanic acid (20/10 µg) disk was placed at the center of the plate while ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg) disks were positioned 20mm from the central disk. The plates were incubated at 37 °C for 18–24 hours. ESBL production was indicated by enhancement or distortion of the zone of inhibition of the cephalosporin disks toward the clavulanic acid containing disk. Phenotypic verification of ESBL production was subsequently performed using the combination disk test. Here, ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg) as well as

cefotaxime (30 µg) and cefotaxime-clavulanic acid (30/10 µg) were applied as previously described. An increase of ≥ 5 mm in the zone of inhibition diameter for the antimicrobial agent combined with clavulanic acid compared with a cephalosporin alone was interpreted as a confirmation of ESBL production. *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as ESBL positive and negative control strains.

Determination of gene constitution of *E. coli* and *K. pneumoniae* isolates

To determine the presence of ESBL production among bacterial isolates, bacterial DNA was extracted from all isolates obtained. Colonies suspended in normal saline were washed once with $1\times$ Tris-EDTA (TE) buffer and centrifuged to obtain a pellet. The resulting pellet was resuspended in TE elution buffer and vortexed for 1 minute. The suspension was then incubated at 95 °C for 1 hour and 30 minutes in a heating dry bath to facilitate cell lysis. The suspension was then centrifuged at 15,000 rpm for 5 minutes, and the supernatant containing genomic DNA was carefully transferred to a new microcentrifuge tube. The extracted DNA was subsequently stored at -20 °C.

Polymerase Chain Reaction (PCR) was performed to detect ESBL genes, including blaCTX-M, blaTEM, blaSHV, and blaOXA [10,11]. Primers targeting ESBL genes were selected from previously validated sequences reported in the literature or designed for use in this study. Each primer pair was designed to amplify specific open reading frames and optimized with respect to sequence specificity, primer concentration, and annealing temperature (Table 1).

PCR amplification was carried out in a conventional PCR Thermocycler (CLASSIC K960 Thermal Cycler). the amplification process included initial denaturation at 95°C for 3 minutes followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 51.2°C for 30 seconds and elongation at 72°C for 1 minute and the final extension cycle of 72°C for 5 minutes. The

DNA amplicon was electrophoresed using 1.5% agarose gel, in 1x Tris-Borate EDTA buffer (TBE), 5 μ L DSVIEW™ Nucleic acid stain (cat. No.: M7011), 6X loading buffer (GDSBio Lot 050), and DNA ladder/marker 100bp (GDSBio Lot 076). Electrophoresis was run at 200V and 80mA for 1 hour. Bands were visualized using the Gene-Flash Trans-illuminator. The results were documented and the gene profiles were recorded for each isolate in a systematic manner.

Data Analysis

The collected phenotypic and genotypic data was analysed using STATA 17 (StataCorp LLC, College Station, TX, USA). Descriptive statistics were used to summarize the resistance patterns, prevalence of ESBL genes, and their distribution. Comparisons of antimicrobial resistance patterns for *E. coli* and *K. pneumoniae* were made using the chi square test. Data presentation was done by tables and figures. Chi Square statistic was used to determine the association between phenotypic and genotypic characteristics.

Quality Control

To ensure the reliability and validity of the data collected during the study, the following were observed. Standardized Protocols were used to ensure all isolates were subjected to similar conditions during susceptibility testing and genomic analysis. Quality-assured reagents, appropriate equipment calibration, regular proficiency testing, and adherence to standard operating procedures was ensured. We established a sample tracking system by assigning a unique identifier to each sample and maintain a detailed record of their handling, storage, and transportation to ensure traceability and to avoid mix-up errors. Appropriate positive and negative controls were included during laboratory testing to validate the accuracy of assays. In addition, we optimized primer concentrations and PCR conditions through pilot experiments to establish appropriate conditions for the experiments.

Ethical Considerations

We obtained ethical clearance and approval from Mbarara University of Science and Technology Research Ethics committee under approval number MUST-2023-1242. Administrative clearance and permission to use stored isolates was obtained from the Principal Investigator of the original study in which samples were collected from communities.

Results

Distribution of *E. coli* and *K. pneumoniae* isolates

A total of 136 bacterial isolates were retrieved, among which 96(70.59%) were *E. coli*. (Fig 1).

Proportion of ESBL production among isolates

The prevalence of ESBL producing isolates was 37.50% (95% CI=29.58 to 46.52). (Table 2).

Distribution of ESBL genes among isolates

Out of 96 *E. coli* isolates tested for ESBL genes, 61.46% of the samples tested positive for blaCTX-M compared to 56.25% for blaTEM. blaOXA and blaSHV genes were least observed in 25% and 27.08% of the isolates respectively. Among the 40 *K. pneumoniae* isolates tested, there was a uniform trend in the distribution of the ESBL genes (Table 3).

Association between phenotypic and genotypic ESBL production

In this study, the presence of blaCTX-M ($X^2=8.61$, $p=0.003$) and blaTEM ($X^2=15.89$, $p=0.001$) genes was associated with a positive phenotypic ESBL characterization. There was no association between phenotypic ESBL production and the blaOXA and blaSHV genes (Table 4).

Discussion

This study identified a notably high prevalence of 37.5% (95% CI: 29.58-46.52) for ESBL production among *E. coli* and *K. pneumoniae* isolates from communities in southwestern Uganda. The observed prevalence aligns with growing concerns about ESBL-mediated resistance in Uganda¹⁵. However, the noteworthy relatively wide 95% confidence interval around the prevalence of ESBL production indicates a moderate precision of the estimate which reflects data variability. In contrast, one other study at Mulago National Referral Hospital (MNRH) found even higher rates of ESBL production at of 62%¹⁶. These findings suggest a widespread issue with ESBL-mediated resistance in Uganda, potentially impacting both human and animal health.

Our study found no significant difference in the distribution of ESBL producers between *Escherichia coli* and *Klebsiella pneumoniae* ($\chi^2 = 0.15$, $p = 0.69$), suggesting that both organisms may play comparable roles as reservoirs and disseminators of ESBL genes in the study setting. Previous studies conducted in Uganda have reported mixed findings regarding the predominant ESBL-producing species^{16,17}. For example, a study conducted at Kabale Regional Referral Hospital reported comparable proportions of *E. coli* (44%) and *Klebsiella* spp. (52%)¹⁸, similar to findings reported in Ethiopia¹⁹, which supports the observations of the present study. However, other Ugandan studies have reported *E. coli* as the predominant ESBL producer, while some have identified *K. pneumoniae* as the dominant species¹⁶. These differences may be attributed to variations in study populations, clinical specimen types, antimicrobial use patterns, and healthcare settings²⁰. The comparable contribution of both organisms to ESBL production highlights the importance of continuous surveillance and strengthened antimicrobial stewardship programs to limit the spread of ESBL-mediated resistance²¹.

In this study, ESBL genes were detected in both *Escherichia coli* and *Klebsiella pneumoniae* isolates. Generally, blaCTX-M and blaTEM were the

most frequently detected ESBL genes, with 61.46% and 56.25% of *E. coli* isolates having blaCTX and blaTEM genes respectively (Table 3). Comparable findings have been reported in Uganda, including at Mulago National Referral Hospital, where blaCTX-M (70%) and blaTEM (34%) were the most frequent ESBL genes^{4,15,22}. Among *K. pneumoniae* isolates, the distribution of ESBL genes appeared more uniform (Table 3), reflecting a potentially diverse genetic profile and supporting previous observations that *Klebsiella* spp. harbors multiple ESBL genes with varying prevalence across geographical regions^{12,23}.

The detection of both blaCTX-M and blaTEM in substantial proportions of *E. coli* and *K. pneumoniae* isolates highlights the risk of horizontal gene transfer, contributing to the dissemination of resistance within and between species²⁴. These findings align with the growing concern over the spread of ESBL-producing Enterobacterales in both healthcare and community settings, particularly in regions with high antimicrobial use, including livestock and environmental reservoirs^{21,25}.

An analysis of the association between phenotypic ESBL production and the presence of specific ESBL genes revealed that isolates with blaCTX-M and blaTEM genes were significantly more likely to express ESBL phenotypes ($\chi^2 = 8.61$, $p = 0.003$ and $\chi^2 = 15.89$, $p = 0.001$, respectively (Table 4). In contrast, the presence of blaOXA ($p = 0.46$) and blaSHV ($p = 0.20$) genes was not significantly associated with phenotypic ESBL gene expression. This finding is consistent with the genetic heterogeneity of the two β -lactamase families, in which only specific variants exhibit extended-spectrum activity while many commonly established alleles such as SHV-1 and most OXA-type enzymes are narrow-spectrum β -lactamases that do not hydrolyze third-generation cephalosporins^{26,27}. Consequently, detection of these genes alone does not reliably predict ESBL phenotype, particularly in settings where CTX-M enzymes predominate as the principal drivers of ESBL production^{28,29}.

These findings demonstrate that blaCTX-M and blaTEM genes remain the primary determinants of ESBL-mediated resistance in both *Escherichia coli* and *Klebsiella pneumoniae* isolates in southwest Uganda, aligning with global trends. Nonetheless, partial discordance between genotype and phenotype highlights the complexity of ESBL mechanisms, emphasizing the importance of integrating both phenotypic and molecular methods in surveillance and antimicrobial stewardship efforts to effectively control the spread of ESBL producing Enterobacterales³⁰.

Considering the farming community settings in which the samples were collected, these findings provide a critical outlook of the distribution of ESBL producing enterobacterales. Farming households constitute a uniquely interconnected ecological systems in which humans, livestock, and the environment are in frequent interaction, creating opportunities for the persistence, exchange, and dissemination of antimicrobial resistant bacteria³¹

Conclusion and Recommendations

This study demonstrates a substantial prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* among community members in southwestern Uganda indicating that clinically important antimicrobial-resistant bacteria are established beyond clinical settings. The predominance of blaCTX-M and blaTEM genes particularly among *E. coli* isolates reflects the continued transmission of ESBL resistance within the community and raises concerns about the effectiveness of commonly used β -lactam antibiotics.

These findings emphasize the need to strengthen community based antimicrobial resistance surveillance to track circulating antimicrobial resistance trends among humans, animals and the environment. Integrating community and clinical surveillance data will improve early recognition of

resistance trends and support more targeted antimicrobial stewardship interventions in similar settings.

Limitations of the study

Authors note the following limitations. Although this study was conducted within a one health setting, comparative analyses of antimicrobial resistance patterns across human, animal, and environmental sources were not undertaken. Thus, these findings may not be used to infer potential transmission pathways of ESBL producing enterobacterales within farming communities. Molecular characterization performed in this study was limited to selected ESBL genes distribution and did not include genome sequencing to identify specific gene variants and as a result, the specific circulating alleles and mechanisms underlying the transmission of ESBL genes could not be fully established.

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Author contributions

All authors contributed equally

Data availability statement

All data available upon reasonable request to the corresponding author. The data is not publicly available due to ethical restrictions.

Competing Interests Statement

Authors declare no competing interests

Tables

Table 1: Characteristics of primers used in the study

Primer	Sequence 5 ¹ -3 ¹	Volume (μL)	Amplicon size (bp)	Annealing temperature (°C)	reference
blaCTX-M	F-CGCTTTGCGATGTGCAG R-ACCGCGATATCGTTGGT	10	550	51.5	32
blaTEM	F-CATTTCGTCGCCCCTTATTC R-CGTTTCATCCATAGTTGCCTGAC	15	800	51.5	32
blaSHV	F-GGTTATGCGTTAATTCGCC R-TTAGCGTTGCCAGTGCTC	10	320	51.5	Primers designed for the first time for use in this study
bla-OXA	F-GATTTGCTCCGTGGCCGAAA R-CCTTGATCGCCCTCGATT	20	281	51.5	21

Table 2: ESBL production among *E. coli* and *K. pneumoniae* isolates

Bacteria isolate	Phenotypic ESBL	Total n (%)

	Positive n (%)	Negative n (%)	
<i>E. coli</i>	37(38.54%)	59 (61.46%)	96 (100)
<i>K. pneumoniae</i>	14 (35.00%)	26 (65.00%)	40 (100)
Total	51 (37.50)	85(62.50%)	136 (100)

Table 3: Prevalence of ESBL genes within *E. coli* and *K. pneumoniae* isolates

ESBL Genes	Positive (%) [95% CI]	Negative (%) [95% CI]	Total
<i>E. coli</i>			
blaCTX-M	59 (61.46%) [50.97 to 71.22]	37 (38.54%) [28.78 to 49.023]	96 (100%)
blaTEM	54 (56.25%) [45.75 to 66.36]	42 (43.75%) [33.64 to 54.25]	96 (100%)
blaSHV	26 (27.08%) [18.52 to 37.11]	70 (72.92%) [62.89 to 81.48]	96 (100%)
blaOXA	24 (25%) [16.72 to 34.88]	72 (75.00%) [65.12 to 83.28]	96 (100%)
<i>K. pneumoniae</i>			
blaCTX-M	15 (37.5%) [22.73 to 54.19]	25 (62.5%) [45.80to 77.27]	40 (100%)
blaOXA	13(32.5%) [18.57 to 49.13]	27 (67.5) [50.87 to 81.43]	40 (100%)
blaTEM	12 (30%) [16.56 to 46.53]	28 (70%) [53.47 to 83.44]	40 (100%)

blaSHV	12 (30%) [16.56 to 46.53]	28 (70%) [53.47 to 83.44]	40 (100%)
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Table 4: The association between ESBL production and ESBL genes carriage among bacterial isolates

Gene	Results	Phenotypic ESBL production		Total (%)	X ²	p. Value
		Positive	Negative			
blaCTX-M	Positive (%)	36 (48.65%)	38 (51.35%)	74(100%)	8.61	0.003*
	Negative (%)	15 (24.19%)	47 (75.81%)	62 (100%)		
blaTEM	Positive (%)	36 (54.55%)	30 (45.45%)	66(100%)	15.89	0.001*
	Negative (%)	15 (21.43%)	55 (78.57%)	70 (100%)		
blaOXA	Positive (%)	12 (32.43%)	25(67.57%)	37 (100%)	0.55	0.46
	Negative (%)	39 (39.39%)	60(60.61%)	99 (100%)		
blaSHV	Positive (%)	11 (28.95%)	27 (71.05%)	38 (100%)	1.65	0.20
	Negative (%)	40 (40.82%)	58 (59.18%)	98 (100%)		

Figures

Fig 1. Distribution of *E. coli* and *K. pneumoniae* isolates

