



OPEN Fecal carriage and molecular characterization of ESBL-producing Enterobacteriaceae among farmers in Mid-Western Uganda

Galimaka Wilson^{1,2✉}, Kabera Micheal¹, Abaasa Catherine¹, Nalumaga Pauline Petra^{1✉}, Fredrickson B. Wasswa², Kassaza Kennedy², Ampaire Lucus¹ & Joel Bazira^{2✉}

Extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae pose a growing threat to public health due to resistance to β -lactam antibiotics. This study aimed to determine the prevalence, antimicrobial resistance patterns, and genetic characterization of ESBL-producing Enterobacteriaceae from stool samples of farmers in Kibimba Parish, Kabarole District, Mid-Western Uganda. A cross-sectional study among 250 farmers involved stool culture, ESBL detection via the double-disc synergy test, PCR for *bla*CTX-M, *bla*TEM, and *bla*SHV genes, and antibiotic susceptibility testing. Data were analyzed using STATA v14.2. The prevalence of ESBL-producing Enterobacteriaceae was 36.4% (91/250) among farmers and 34.29 (107/312) (95%CI: 29.22–34.76) among isolated organisms. *Escherichia coli* was the predominant ESBL-producing isolate at 70.4% (76/107). High resistance was observed to piperacillin (94.0%) and ampicillin (81.4%), while imipenem (96.5%) and chloramphenicol (94.0%) showed the highest susceptibility. Multidrug resistance (MDR) was noted in 49.4% of all isolates and 33.7% of ESBL producers. Among 107 confirmed ESBL producers, 80.4% harbored at least one ESBL gene; *bla*CTX-M (48.7%), *bla*TEM (34.9%) and *bla*SHV (16.4%). ESBL carriage was significantly associated with use of shallow well water ($p < 0.009$), goat farming ($p < 0.036$), and chronic illness ($p < 0.012$). Farmers showed high fecal carriage of multidrug-resistant ESBL-producing Enterobacteriaceae, linked to environmental and health-related risk factors.

Keywords Antimicrobial resistance, AMR surveillance, Enterobacteriaceae, Extended-spectrum β -lactamases, One health, Rural Uganda

Extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae are a significant global public health threat due to their ability to inactivate a wide range of β -lactam antibiotics, including third- and fourth-generation cephalosporins and monobactams^{1–4}. These enzymes are encoded by genes such as *bla*CTX-M, *bla*TEM, and *bla*SHV, which are often plasmid-borne and can be horizontally transferred across bacterial species⁵. Infections caused by ESBL-producing bacteria are associated with limited therapeutic options, increased morbidity, and higher healthcare costs^{6–9}.

The burden of antimicrobial resistance (AMR), particularly due to ESBLs, is exacerbated in low- and middle-income countries (LMICs), where the misuse of antibiotics in both human and animal health is prevalent^{10,11}. The “One Health” framework recognizes that AMR transcends human healthcare settings and involves environmental, animal, and agricultural domains¹². Evidence increasingly shows that food-producing animals, such as poultry and livestock, may serve as reservoirs for ESBL-producing bacteria, facilitating transmission through direct contact, contaminated food, or environmental exposure^{13,14}.

In Uganda, antibiotic use in livestock farming is widespread and often unregulated, contributing to the emergence and spread of resistant bacterial strains^{15,16}. To date, limited data exist on the fecal carriage and genetic profile of ESBL-producing Enterobacteriaceae among farming communities in Uganda. This study aimed to determine the prevalence, antimicrobial resistance patterns, and molecular characteristics of ESBL-producing Enterobacteriaceae in fecal samples from farmers in Kibimba Parish, Kabarole District, Mid-Western Uganda.

¹Faculty of Medicine, Department of Medical Laboratory Sciences, Mbarara University of Science and Technology, P. O. Box 1410, Mbarara, Uganda. ²Faculty of Medicine, Department of Microbiology, Mbarara University of Science and Technology, Mbarara, Uganda. ✉email: galimakaw@gmail.com; ppaulinenalumaga@must.ac.ug; jbazira@must.ac.ug

Understanding these dynamics is essential for designing effective interventions and guiding antimicrobial stewardship efforts within the One Health framework.

Materials and methods

Study design and setting

A descriptive cross-sectional study was conducted between September and November 2022 among farmers in Kibimba Parish, West Division, Kabarole District, Mid-Western Uganda. This rural area is predominantly agricultural, with common practices in smallholder livestock and poultry farming. Stool sample collection and culture were performed at Fort Portal Regional Referral Hospital (FRRH), a government-funded and SANAS-accredited facility. Molecular analyses were conducted at DC Laboratories in Kamukuzi, Mbarara.

Study population and sample size

Farmers were recruited using a purposive sampling strategy. Participants were eligible if they were actively involved in farming, aged 18 years or older, and consented to participate. A sample size of 250 was determined based on ESBL prevalence of 17%¹⁷; using the Kish and Leslie formula (1965) with a 95% confidence level and 5% margin of error and 15.5% contingency for potential data loss. While sampling was not randomized, efforts were made to include a broad representation of smallholder and livestock farmers across selected villages to ensure variability in exposure and farming practices.

Sample collection and handling

Each participant provided a fresh stool sample (~ 1 g) in a sterile, leak-proof container. Samples were labeled with unique IDs and demographic details, then transported under cold chain (4 °C) to FRRH microbiology laboratory for immediate processing within 4 h to preserve integrity and minimize contamination. All procedures were performed in accordance with the relevant guidelines and regulations.

Laboratory procedures

Culture and identification

Stool samples were cultured on Xylose Lysine Deoxycholate (XLD) agar, MacConkey agar, and Nutrient agar (Oxoid Ltd., UK)¹⁸. XLD was included to screen for enteric pathogens like Salmonella, while MacConkey served as the primary medium for isolating lactose- and non-lactose-fermenting Enterobacteriaceae. Nutrient agar was used for subculturing and preparing pure isolates for molecular characterization.

The plates were incubated for 18–24 h at 37 °C. Morphologically distinct colonies were selected based on color and fermentation status. Preliminary identification of Enterobacteriaceae isolates was performed through Gram staining, followed by a series of standard biochemical tests. The Simmons citrate test was used to assess the ability of isolates to utilize citrate as a sole carbon source. Triple Sugar Iron (TSI) agar was employed to evaluate carbohydrate fermentation (glucose, lactose, and sucrose) and to detect the production of hydrogen sulfide (H₂S). Additionally, the Sulphide Indole Motility (SIM) test was used to assess H₂S production, indole formation, and motility¹⁸.

Antimicrobial susceptibility testing (AST)

AST was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar following CLSI 2021 guidelines¹⁹. The bacterial suspension was adjusted to 0.5 McFarland standard and inoculated onto the agar surface. Antibiotics (Oxoid Ltd UK, Lot. ch.b 3350743 and Bio-analyse Ltd Turkey, Lot. 221011 A), tested included: β -lactams: Cefotaxime (30 μ g), Cefoxitin (30 μ g), Ceftazidime (30 μ g), Ceftriaxone (30 μ g), Cefepime (30 μ g), Ampicillin (10 μ g), Piperacillin, Amoxicillin-clavulanic acid (20/10 μ g), Aztreonam (10 μ g); Carbapenems: Imipenem (10 μ g); Non- β -lactams: Chloramphenicol (30 mg), Ciprofloxacin (5 μ g), Gentamicin (10 μ g), Trimethoprim-Sulfamethoxazole (25 μ g), Azithromycin (15 μ g).

Isolates resistant to third-generation cephalosporins were screened for potential ESBL production³. Confirmatory testing was done using the Double Disc Synergy Test (DDST) with Ceftazidime and Cefotaxime (30 μ g each) placed near Amoxicillin-clavulanic acid (20/10 μ g)¹. Confirmatory testing and antimicrobial susceptibility testing (AST) were performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following CLSI guidelines (2021).

Quality control was ensured using *E. coli* ATCC 25,922 and *K. pneumoniae* ATCC 700,603 as reference strains. Sterility checks were performed using uninoculated media controls.

DNA extraction and PCR amplification

Genomic DNA was extracted using the boiling lysis method. PCR was performed to detect ESBL-associated genes (*bla*CTX-M, *bla*TEM, and *bla*SHV) using specific primers (Table 1). PCR reactions were prepared using Hot Start Taq 2x Master Mix (New England Biolabs) in a 25 μ L reaction volume. Amplification conditions included an initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation (95 °C for 45s), annealing (52–55 °C), extension (72 °C for 1 min), and a final extension at 72 °C for 5 min. PCR validation included positive control strains known to harbor each gene, and nuclease-free water as a negative control. Amplicons were resolved by electrophoresis on 1.5% agarose gels stained with SafeView™ and visualized under UV light.

Data management and statistical analysis

Data were entered into Microsoft Excel and exported to STATA version 14.2 for analysis. Descriptive statistics were used for prevalence and resistance patterns. Descriptive statistics were used to compute prevalence estimates and proportions, with results reported alongside 95% confidence intervals (CIs). Group comparisons

Genes	Primers	Sequence (5' to 3')	Amplicon size (bp)	Reference
<i>bla</i> _{SHV}	Forward Reverse	F: 5'ATGCGTTATATTCGCCTGTG-3' R: 5'-TGCTTTGTTATTCGGGCCAA-3'	747	20
<i>bla</i> _{TEM}	Forward Reverse	F: 5'-CATTTCGTTGTCGCCCTTATTC-3' R: 5'-CGTTCATCCATAGTTGCCTGAC-3'	800	Younis et 2017
<i>bla</i> _{CTX-M}	Forward Reverse	F: 5'-CGCTTTGCGATGTGCAG-3' R: 5'-ACCGCGATATCGTTGGT-3'	590	21

Table 1. PCR primers and amplicon size used in PCR reaction.

Socio-demographic factors	Category	Freq (N= 250).	Percent (100.00%)
Age group	20-29yrs	48	19.20
	30-39yrs	58	23.20
	40-49yrs	87	34.80
	≥ 50yrs	57	22.80
Gender	Male	126	50.40
	Female	124	49.60
Education level	Primary	130	52.00
	Secondary	60	24.00
	Tertiary	24	9.60
	University	4	1.60
Marital status	Non-formal	32	12.80
	Single	35	14.00
	Married	198	79.20
Occupation	Divorced	6	2.40
	Widow	11	4.40
	Peasant	168	67.20
	Civil servant	55	22.00
	Business	13	5.20
Employment status	Student	1	0.40
	Others(driver, boda-boda riders, market vendors)	13	5.20
	Employed	67	26.80
	Self-employed	115	46.00
Farming as the only source of income	Unemployed	68	27.20
	Yes	120	48.00
Animal family ownership	No	130	52.00
	Yes	179	71.60
	No	71	28.40

Table 2. Socio-demographic characteristics of participants.

were assessed using the Chi-square test. To determine factors independently associated with ESBL carriage, binary logistic regression was performed. Variables with a p-value ≤ 0.1 in bivariate analysis were included in the multivariate model. Associations were considered statistically significant at $p \leq 0.05$, and findings are presented as adjusted odds ratios (aORs) with corresponding 95% CIs.

Results

Socio-demographic characteristics of study participants

A total of 250 farmers participated in the study, yielding a 100% response rate. The majority were aged above 40 years (57.6%), with a nearly equal distribution of males (50.4%) and females (49.6%). Most participants had attained at least a primary level of education (52%), were married (79.2%), and engaged in peasant farming (67.2%). Notably, 71.6% of participants reported owning animals as shown in Table 2 below.

Distribution of isolates

Out of the 250 stool samples collected, 249 yielded bacterial growth, from which 312 Gram-negative isolates were recovered. *Escherichia coli* was the most frequently isolated organism, accounting for 70.2% (219/312) of all isolates, followed by *Citrobacter spp.* (6.7%), *Klebsiella pneumoniae* (4.8%), *Klebsiella oxytoca* (3.8%), and *Enterobacter spp.* (4.2%). Rare isolates included *Shigella spp.*, *Proteus vulgaris*, *Salmonella spp.*, and *Enterobacter aerogenes* (each < 2%). see Table 3.

Isolate	Frequency	Percentage (%)
<i>Escherichia coli</i>	219	70.2
<i>Klebsiella pneumoniae</i>	15	4.8
<i>Klebsiella oxytoca</i>	12	3.8
<i>Citrobacter freundii</i>	06	2.0
<i>Citrobacter koseri</i>	15	4.8
Other <i>Citrobacter</i> spp	21	6.7
<i>Enterobacter cloacae</i>	01	0.3
<i>Enterobacter</i> spp	13	4.2
<i>Shigella</i> spp	04	1.3
<i>Salmonella paratyphi A</i>	01	0.3
<i>Salmonella</i> spp	01	0.3
<i>Proteus vulgaris</i>	03	1.0
<i>Enterobacter aerogenes</i>	01	0.3
Total	312	100

Table 3. Proportion of the isolated gram negative organisms from farmers.

Isolate	ESBL production		
	Non-producer % (n)	ESBL-producer % (n)	Total % (n)
<i>Escherichia coli</i>	70.0 (143)	70.4(76)	70.2(219)
<i>Klebsiella pneumoniae</i>	5.9 (12)	2.8(3)	4.8(15)
<i>Klebsiella oxytoca</i>	3.4 (7)	4.6 (5)	3.8(12)
<i>Citrobacter freundii</i>	2.0 (4)	1.8 (2)	2.0(6)
<i>Citrobacter koseri</i>	4.4 (9)	5.5(6)	4.8(15)
Other <i>Citrobacter</i> spp	6.8 (15)	6.5 (6)	6.7 (21)
<i>Enterobacter clocae</i>	0 (0)	0.9 (1)	0.3(1)
<i>Enterobacter</i> spp	4.4 (9)	3.7 (4)	4.2(13)
<i>Shigella</i> spp	1.5 (3)	0.9 (1)	1.3 (4)
<i>Salmonella paratyphi A</i>	0.5 (1)	0 (0)	0.3(1)
<i>Salmonella</i> spp	0.5 (1)	0 (0)	0.3(1)
<i>Proteus vulgaris</i>	0 (0)	2.8 (3)	1.0(3)
<i>Enterobacter aerogenes</i>	0.5 (1)	0 (0)	0.3 (1)
Total	100 (205)	100 (107)	100 (312)

Table 4. Distribution of ESBL producers among isolated organisms.

Prevalence of ESBL-producing Enterobacteriaceae

The overall prevalence of ESBL-producing Enterobacteriaceae was 36.4% (91/250 participants), and 34.29% (107/312) (95%CI: 29.22–34.76) among the isolated organism. Among the 107 confirmed ESBL producers, *E. coli* (70.4%), was dominant, followed by *Citrobacter* spp. (6.5%), *Citrobacter koseri* (5.5%), *Klebsiella oxytoca* (4.6%), and *Enterobacter* spp. (3.7%). See Table 4.

Village-level distribution of ESBL isolates

The highest number of ESBL-positive isolates originated from Kibimba A (43.9%), followed by Kibimba B (21.5%) and Muhoti-Kasojo (19.6%). Fewer ESBL producers were found in Kitere (9.3%) and Katojo-Kasindikwa (5.6%).

Antibiotic susceptibility testing of isolated organisms

The antibiotic susceptibility testing revealed a concerning pattern of resistance among the isolated Enterobacteriaceae. Piperacillin and ampicillin showed the highest resistance rates, at 94.0% and 81.4% respectively. Additionally, over one-third of isolates demonstrated resistance to third-generation cephalosporins such as cefotaxime (39.1%), ceftriaxone (35.9%), and ceftazidime (35.3%), suggesting a high prevalence of ESBL phenotypes. Trimethoprim-sulfamethoxazole (51.3%) and amoxicillin-clavulanic acid (67.6%) also showed considerable resistance, further limiting first-line treatment options. In contrast, imipenem (0.3%), chloramphenicol (6.4%), *Piperacillin-Tazobactam* (17.6%), *Cefoxitin* (20.5%), and azithromycin (23.4%) exhibited the lowest resistance levels, with imipenem emerging as the most consistently effective agent as shown in Table 5.

Antibiotic	Susceptibility pattern	
	Sensitive % (n)	Resistant % (n)
CRO	61.5 (191/312)	38.5 (121)
CAZ	59.6 (186/312)	35.3 (126)
CTX	50.6(158/312)	49.4 (154)
FEP	48.7 (152/312)	35.6 (111/312)
FOX	79.5 (248/312)	20.5 (64/312)
IPM	96.5 (301/312)	0.3 (1/312)
ATM	64.4 (201/312)	35.6(111/312)
AMP	18.6(58/312)	81.4(254/312)
AMC	32.4(101/312)	67.6(211/312)
PRL	6.1(19/312)	94.0(293/312)
TPZ	65.7(205/312)	34.3(107/312)
C	94.0(292/312)	6.4(20/312)
CN	56.1(175/312)	43.9(137/312)
CIP	58.0(181/312)	42.2(131/312)
SXT	48.7(152/312)	51.3(160/312)
AZM	76.6(239/312)	23.4(73/312)

Table 5. Antibiotic susceptibility pattern of selected antibiotics. Key: CRO-Ceftriaxone, CAZ-Ceftazidime, CTX-Cefixime, FEP-Cefipime, FOX-Cefoxitin, IPM-Imipenem, ATM-Aztreonam, AMP-Ampicillin, AMC-Amoxicilli, PRL-Piperacillin, TPZ-Piperacillin-Tazobactam. C-Chloramphenicol, CN-Gentamycin, CIP-Ciprofloxacin, SXT-Trimethoprim-Sulphurmethoxazole and AZM-Azithromycin.

Isolated organism	MDR n (%)
<i>Echerichia coli</i>	106 (68.8%)
<i>Klebsiella pneumonia</i>	5 (3.2%)
<i>Klebsiella oxytoca</i>	5/154 (3.2%)
<i>Citrobacter freundii</i>	3/154 (1.9%)
<i>Citrobacter koseri</i>	10 (6.5%)
<i>Citrobacter spp</i>	14 (9.1%)
<i>Enterobacter spp</i>	6 (3.9%)
<i>Shigella spp</i>	2 (1.3%)
<i>Proteus vulgaris</i>	3 (1.9%)

Table 6. The MDR of isolated organisms.

Multidrug resistance (MDR) and MAR index

Multidrug resistance (MDR), defined as resistance to ≥ 3 antibiotic classes, was observed in 49.4% (154/312) of all isolates and 33.7% of ESBL producers. *E. coli* accounted for 68.8% of MDR strains, followed by *Citrobacter spp.* (9.1%) and *Klebsiella spp.* (6.4%). See Table 6.

Distribution of isolates with MAR index ≥ 0.2

The multiple antibiotic resistance (MAR) index was ≥ 0.2 in 76.6% (239/312) of isolates. *E. coli* accounted for the majority (69.5%), followed by *Citrobacter spp.* (8.4%), *Klebsiella pneumoniae* (5.4%), and *Citrobacter koseri* (5.4%). Lower contributions were observed from *Enterobacter spp.* (3.8%), *K. oxytoca* (2.9%), and *Proteus vulgaris* (1.3%). Rare contributors included *Shigella spp.*, *Enterobacter cloacae*, and *Salmonella spp.* (each < 1%). No isolate from *Salmonella paratyphi A* or *E. aerogenes* exhibited a high MAR index.

Proportion of the studied beta-lactamase genes among phenotypically confirmed ESBLs-producing organisms

Among 107 phenotypically confirmed ESBL producers, 80.4% (86/107) were PCR-positive for one or more resistance genes, yielding a total of 152 gene detections. The most common was *bla*CTX-M (48.7%) followed by *bla*TEM (34.9%) and *Bla*SHV 16.4%.

Distribution of studied beta-lactamase genes across species

E. coli harboured the majority of ESBL genes, including *bla*CTX-M 35.5% (54/152), *bla*TEM 26.3% (40/152) and *bla*SHV 13.2% (20/152). Other organisms, including *Klebsiella spp.*, *Citrobacter spp.*, and *Proteus spp.*, contributed lower frequencies of each gene. Table 7.

Isolate	ESBL genes			Total % <i>(n)</i>
	CTX % <i>(n)</i>	TEM% <i>(n)</i>	SHV % <i>(n)</i>	
<i>Escherichia coli</i>	35.5(54)	26.3(40)	13.2(20)	5(114)
<i>Klebsiella pneumoniae</i>	2.0(3)	0.7(1)	0.7(1/152)	3.3(5)
<i>Klebsiella oxytoca</i>	2.6(4)	2.6(4)	0(0/152)	5.3(8)
<i>Citrobacter freundii</i>	0.7(1)	2.0(3)	0.7(1/152)	5.3(8)
<i>Citrobacter koseri</i>	2.6(4)	2.0(3)	0.7(1/152)	5.3(8)
<i>Citrobacter</i> spp	2.6(4)	2.0(3)	0.7(1/152)	5.3(8)
<i>Enterobacter</i> spp	1.3(2)	0(0)	0(0/152)	1.3(2)
<i>Enterobacter cloacae</i>	0.7(1)	0.7(1)	0(0/152)	1.3(2)
<i>Enterobacter aerogenes</i>	0(0)	0(0)	0(0/152)	0(0)
<i>Proteus vulgaris</i>	1.3(2)	0.7(1)	0.7(1/152)	2.6(4)
<i>Shigella</i> spp	0.7(1)	0(0)	0(0/152)	0.7(1)

Table 7. Distribution of studied beta-lactamase gene across species.

Factors	Variable	Bivariate reg. analysis (cOR, 95%CI, P-value)	Multivariate reg. analysis (aOR, 95%CI, P-value)
Socio-demographic factors	Education level	0.44; 0.781–3.346; $p=0.011$	
	Marital status	1.35; 0.652–2.782; $p=0.165$	
	Occupation	0.68; 0.365–1.254; $p=0.115$	
	Employment status	0.11; 0.049–0.241; $p=0.000$	
Environmental factors	Source of water	2.84; 1.349–5.965; $p=0.006$	Shallow well: 4.34; 1.441–13.066; $p=0.009$
	Type of water gathering	1.88; 0.911–3.904; $p=0.012$	
	Type of farming	0.5; 0.240–1.397; $p=0.036$	Goat farming: 0.13; 0.019–0.873; $p=0.036$
	Hygiene of the farm	2.96; 0.688–12.701; $p=0.145$	
Medical factors	Chronic disease	11.64; 1.096–123.703; $p=0.042$	HTN: 14.25; 2.974–64.644; $p=0.012$ HIV/AIDS: 47.74; 1.333–171.703; $p=0.033$
	Use of medication	10.30; 0.877–121.010; $p=0.064$	

Table 8. Factors associated with ESBL among farmers. Significant values are in bold.

Level of occurrence of studied ESBL

Among the 86 PCR-confirmed ESBL-producing isolates, single-gene carriage was most frequently observed with *bla*CTX-M (29.1%), followed by *bla*TEM (7.0%) and *bla*SHV (3.5%). Co-existence of multiple genes was common, with *bla*CTX-M+*bla*TEM detected in 34.9% of isolates being the most frequent combination. A smaller proportion harbored *bla*CTX-M+*bla*SHV (5.8%) or *bla*TEM+*bla*SHV (3.5%). Notably, triple gene co-occurrence (*bla*CTX-M+*bla*TEM+*bla*SHV) was found in 16.3% of isolates, suggesting extensive horizontal gene transfer and the presence of multidrug-resistant genetic platforms in the study population.

Factors associated with ESBL among farmers

Multivariate logistic regression analysis identified several significant predictors of ESBL carriage. Farmers who used shallow well water had significantly higher odds of ESBL colonization (aOR=4.34; 95% CI: 1.44–13.07; $p=0.009$). Interestingly, those engaged in goat farming had lower odds of ESBL carriage (aOR=0.13; 95% CI: 0.019–0.87; $p=0.036$). The presence of chronic health conditions was also associated with increased risk. Specifically, participants with hypertension (aOR=14.25; 95% CI: 2.97–64.64; $p=0.012$) and HIV/AIDS (aOR=47.74; 95% CI: 1.33–171.70; $p=0.033$) were significantly more likely to harbor ESBL-producing organisms. Surprisingly, even individuals without any chronic illness showed elevated odds (aOR=49.30; 95% CI: 1.36–179.27). No statistically significant associations were found with age, sex, education level, or prior hospitalization. See Table 8.

Discussion

This study revealed a high prevalence (36.4%) of ESBL-producing Enterobacteriaceae among farmers in a rural Ugandan community, underscoring the expanding presence of antimicrobial resistance (AMR) beyond hospital settings. *Escherichia coli* was the predominant isolate and ESBL producer, consistent with studies conducted in Ethiopia, Nigeria, and Uganda, and other African countries where *E. coli* remains the principal reservoir of ESBLs in both clinical and community contexts^{3,22–24}.

The resistance patterns observed in this study raise significant public health concerns. Over 90% of isolates were resistant to piperacillin, and more than 80% to ampicillin, highlighting the diminishing effectiveness of commonly used β -lactam antibiotics in rural and agricultural settings. These high resistance levels may reflect widespread, and often unregulated, use of broad-spectrum antibiotics in both human and veterinary medicine. Furthermore, resistance to third-generation cephalosporins, which serve as critical markers for ESBL

production, was recorded in over 35% of isolates a prevalence consistent with earlier studies from Sub-Saharan Africa that report a rising ESBL burden in community settings^{25,26}. In contrast, imipenem (0.6% resistance) remained highly effective, likely due to restricted use and higher cost, a finding echoed in related studies^{27,28}.

The high prevalence of multi-drug resistance (49.4%) and elevated MAR indices (≥ 0.2 in 76.6% of isolates, particularly *E. coli*) supports the presence of significant antibiotic pressure in the study community, consistent with findings from similar settings^{29–31}. High MAR indices suggest exposure to unregulated antibiotic use, possibly linked to veterinary or self-medication, an observation consistent with prior findings in livestock-keeping households in Uganda and other low-income settings^{1,32}.

Molecular screening showed that 80.4% of phenotypically confirmed ESBL producers harboured one or more of the studied resistance genes, with *bla*CTX-M being most prevalent, followed by *bla*TEM and *bla*SHV. This is consistent with global shifts indicating CTX-M dominance over TEM and SHV^{33–35}. The frequent co-occurrence of multiple ESBL genes in this study indicates active horizontal gene transfer likely mediated by mobile genetic elements³⁶. Similar patterns have been observed in studies from Burkina Faso and Ethiopia^{32,37}. These findings have critical public health implications, especially in resource-limited settings where treatment options are restricted and diagnostic capacity is limited.

Notably, several risk factors reported in this study were significantly associated with ESBL carriage. The use of shallow well water was strongly linked to colonization, likely due to contamination from livestock and environmental runoff. This finding aligns with other One Health-based studies that identify unprotected water sources as AMR hotspots^{1,38}. Goat farming was inversely associated with ESBL presence, possibly due to behavioral and housing differences compared to cattle or poultry farming. Chronic illnesses such as HIV/AIDS and hypertension were also significant predictors, likely reflecting immune compromise and repeated antibiotic exposure, as previously observed in many global cohorts^{39,40}.

While these findings highlight the interconnected factors contributing to ESBL transmission and its public health importance in rural communities, the study has several limitations. The cross-sectional design restricts the ability to infer causal relationships between antibiotic exposures and bacterial colonization. Additionally, only human stool samples were analyzed, preventing direct assessment of potential zoonotic or environmental reservoirs. Molecular screening was limited to three major ESBL gene families, potentially underestimating the diversity of resistance mechanisms present. Nonetheless, this study provides essential baseline data for rural Uganda and reinforces the urgent need for integrated antimicrobial resistance surveillance under a One Health framework.

Conclusion

The high fecal carriage of ESBL-producing Enterobacteriaceae among farmers highlights a growing public health threat in rural Uganda. These findings emphasize the need for integrated One Health surveillance across human, animal, and environmental sectors. Promoting hygiene, educating farmers on antibiotic use, and strengthening antimicrobial stewardship are essential to curb the spread of resistance. Targeted interventions and improved diagnostic capacity in rural settings will be critical for early detection and control of AMR.

Data availability

The raw data of this study are available on request to the corresponding author.

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Author contributions

Galimaka Wilson (GW), Kabera Micheal (KM),- Conceptualization, Data collection Nalumaga Pauline Petra(NPP), Wasswa Fredrickson. B (WFB), Kassaza Kennedy (KK), GW- Laboratory processesAmpaire Lucus (AL) Joel Bazira (JB), Abaasa Catherine (AC)- Supervision; Administration.GW, KM, KK, WFB- manuscript draftingAL, JB, AC, NPP- Editing and final manuscript writing.

Declarations

Competing interests

The authors declare no competing interests.

Ethical considerations

Ethical approval was obtained from the Mbarara University Research Ethics Committee (REC Ref: MUST-

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Additional information

Correspondence and requests for materials should be addressed to G.W., N.P.P. or J.B.

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