

Profiles of diarrheagenic *E. coli* Pathotypes, *Salmonella*, and Extended Spectrum β -Lactamase resistance genes in Diarrheal Cases at Ruhengeri Level 2 Teaching Hospital, Rwanda

Yoranda Imurinde

yimurinde@gmail.com

INES-Ruhengeri

Celestin Musabyumuremyi

INES-Ruhengeri

Liliane Muhimpundu

Jomo Kenyatta University of Agriculture and Technology

Ezechiel Bizimana

University of Global Health Equity

Hyacinthe Mukashema

INES-Ruhengeri

Pierre Mujiyarugamba

Manchester Royal Infirmary

Thiery Habyarimama

INES-Ruhengeri

Bruno Niyonkuru Rugira

INES-Ruhengeri

Joseph Mucumbitsi

Adventist University of Central Africa

Callixte Yadufashije

INES-Ruhengeri

Research Article

Keywords: *Escherichia coli*, *Salmonella*, antimicrobials, Extended Spectrum β -lactamases (ESBL)

Posted Date: June 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9416218/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Escherichia coli and *Salmonella* are recognized as gastrointestinal infection causative agents, and present a significant burden in multidrug-resistance, resulting from their capacity to produce Extended-Spectrum Beta-Lactamases (ESBLs) enzyme, which inhibits the drug action and influence drug resistance.

Methods

This was a cross-sectional study carried out from February to July 2025. A total of 133 diarrheal stool samples were collected and cultured to identify *E. coli* and *Salmonella* pathotypes. Antibiotic susceptibility testing was performed using the disc diffusion method. Phenotypic determination of ESBL production was tested using the double disc synergy test. Colony PCR was used to detect ESBL genes (blaCTX-M, blaSHV, and blaTEM).

Result

E. coli pathotypes were the predominant isolates, with Enterotoxigenic *E. coli* (ETEC) and Enteropathogenic *E. coli* (EPEC) being the most frequent and stood at 67.9% and 22.4% respectively. The prevalence of *Salmonella enterica* was 13.8%. High resistance to ampicillin was observed, while meropenem remained effective to both isolates. blaSHV was the most common ESBL gene (70%), followed by blaCTX-M (30%), and the least was blaTEM (20%).

Conclusion

The findings highlight a high prevalence of diarrheagenic *E. coli* pathotypes and *Salmonella in diarrhea patients*, emphasizing the urgent need for routine testing both bacteria as pathogen regardless the location, it will help in implementation of antimicrobial stewardship program in clinical settings.

INTRODUCTION

Diarrhea remains one of the most significant public health challenges globally, particular in low and middle income countries, where it counts for an estimated 1.3 million death annually and continues to substantially contribute to morbidity and mortality [1], [2]. It is responsible for approximately 2.6% of the total disease burden [3]. Clinically, diarrhea is characterized by the passage of loose or watery stool and is frequently caused by bacteria, virus, parasites infections. The etiological agents vary depending on geographical region, where *Escherichia coli* and *Salmonella* species are the most common causative bacteria [4]. *E. coli* strains that cause diarrhea are divided into six major groups based on their virulence traits:

diffuse-adherent *E. coli*, enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) [5]. *Salmonella* is classified into two species, enterica, and bongori [6].

Globally, diarrhea continue to impose a substantial health burden, in 2021, it caused an estimated 1.17 million deaths with 52% affecting children under five years of age [3], [2], [7]. In Africa particularly Western Africa, diarrhea mortality was high in all ages, with 194.5 deaths per 100,000 in children under five years but for children above five years 33.5 deaths per 100,000 [8]. The studies conducted in East Africa showed that diarrheal is a big concern [2]. In Burundi, the results showed that 32.6% of cases were reported among children < 5 years old [9]. The prevalence of diseases in Tanzania was 11.6% [10]. In Rwanda, the prevalence of diarrhea among children under five was reported and observed to be higher in this group of age compared to others [11]. Enterotoxigenic *Escherichia coli* was estimated to be the most common bacteria that causes diarrhea in Rwanda [12]. 95% of the diarrheal cases in the entire African region were caused by non-typhoidal *Salmonella* spp. [8].

Antimicrobial resistance (AMR) is a growing and active pandemic worldwide, particular in enteric pathogens that serves as critical environment for the emergence and transmission of multidrug-resistant organisms (MDROs) [13]. One of the key contributors to this resistance is the production of Extended Spectrum β -lactamases (ESBL), are a group of enzymes that cause resistance to β -lactam antibiotics, although inhibitors like clavulanic acid can neutralize these enzymes but the rapid evolution of resistance genes limits their long-term effectiveness [14].

Numerous studies have highlighted the widespread dissemination of Extended spectrum beta - lactamase producing strains of *Escherichia coli* and *Salmonella* throughout human, livestock, and aquaculture sectors, which may be a factor in persistence and challenging-to-treat diarrheal illnesses [15]. These multi-drug resistance strain of *E. coli* and *Salmonella* are capable of causing of both intestinal and extra-intestinal infections. Among them *E. coli* has shown a notable high rate of multi-drug resistance, exhibiting a strong resistance to antibiotics such as penicillin and ceftazidime, while demonstrate relatively low resistance to gentamycin [16]. Similarly, a recent study from India revealed that 28% of non-typhoidal *salmonella* isolates were multi-drug resistant (MDR) [17].

The spread of antibiotic resistance is largely driven by the presence of ESBL-encoding genes [18]. Currently, 4400 known β -lactamases and new ones are constantly being discovered all around the world, CTX-M is ESBL that is commonly prevalent [19]. A large majority of *E. coli* (91.8%) carried blaCTX-M alone or in combination with other ESBL genes such as blaSHV, and blaTEM (1.8%) [20]. In diarrheagenic *E. coli* isolates, blaTEM was the only resistance gene detected and only 6% of *Salmonella* isolates carried such gene [21].

In resource-limited settings, distinguishing between pathogenic and commensal strains of *E. coli* and *Salmonella* remains a significant challenge, hindering proper diagnosis and treatment. Therefore, it is crucial to characterize the pathogenic strains and determine the presence of ESBL-resistance genes. This study aims to conduct molecular analysis of *E. coli* and *Salmonella* pathotypes to identify ESBL-

producing strains in diarrheal cases. The findings are expected to enhance understanding of local antimicrobial resistance patterns and contribute to improved public health interventions and antimicrobial stewardship efforts in Rwanda and beyond.

METHODS

Study area

The study was conducted at Ruhengeri Level 2 Teaching hospital in Musanze district, Northern Province of Rwanda and it provide a compressive health care services to inpatients as well as outpatients. Data collection took place from February to July, while Laboratory analysis was performed at the bacteriology of INES-Ruhengeri University.

Study design

The study utilized a cross-sectional design to collect and analyze the data.

Study population

Around 133 adult and children with diarrhea symptom was enrolled in this study. Participation was limited to those who gave consent, while individual on antibiotics, unable to provide a diarrhea stool sample, and who did not consent were excluded.

Sampling and sample size

Purposive sampling method was used to select patients with diarrhea symptom. The Yamane formula was used to determine the sample size to ensure a statistically significant representation of the patient population: $n = N / (1 + N * e^2)$, where n is the sample size, N is the population size (200), and e is the margin error (0.05). According to the Yamane formula, $n = 200 / (1 + 200 * 0.05^2)$, the sample size of this study was 133.

Specimen collection

Diarrhea stool samples (20-40mgs) were collected aseptically from each consenting participant in sterile, leak-proof containers and transported immediately to the bacteriology laboratory at INES-Ruhengeri university in cooler box. Upon arrival, samples were processed within 2–4 hours to ensure viability of *E. coli* and *Salmonella* pathogens.

Inclusion criteria

The research focuses exclusively on diarrhea stool samples from individuals with diarrhea symptoms.

Exclusion criteria

Patients who were currently using antibiotics were excluded, and certain types of stools like blood stool samples were not included in the study.

Laboratory techniques

Diarrhea stool samples were processed for the isolation and identification of *E. coli* and *Salmonella* using selective culture media, including MacConkey and XLD, with pre-enriched and enriched steps for *Salmonella*. Colonies were identified through gram staining and biochemical tests (**IMViC**), and *E. coli* serotypes were confirmed via slide agglutination with specific O and H antisera. Antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following CLSI version 2025 guidelines for interpretation. ESBL production was phenotypically confirmed using the Combined Disk Synergy test, and ESBL positive isolates was extracted via boiling method, after DNA was amplified in Thermocycler machine (1h45min) by using forward and reverse primers in the following conditions: initial denaturation was at 95⁰C for 5min, final denaturation was at 95⁰C for 20sec, annealing was at 61⁰C for 30sec and extension was at 72⁰C for 1min to make 40 cycles as well as final extension was at 72⁰C for 7min (1) with amplification products visualized on 2% agarose gel stained with ethidium bromide.

Data and statistical analysis

Data and statistical analysis were summarized and presented in form of figures and tables. IBM SPSS version 21 was used for data analysis.

RESULT

Demographic characteristics of study participants

The participants were all patients with diarrhea symptom. The age group which was the most predominant was 0–10 (32.1%), followed by age groups > 55(26.1%), 11–21 (19.4%), 22–32 (8.2%) respectively while the least age groups 33–43 and 44–54 had equal participation with 6.7%. Female had a high number of participants with 85 (63.4%) compared to male with 48 (35.8). Based on occupation, students were highly participated in this study with 62 (46.3%), unemployed with 30 (22.4%), farmer 26 (19.4%), others job with 15 (11.2%) respectively. The participant living in rural region were participated higher compared to those living in urban with 76 (56.7%), and 57 (42.5%) respectively. Regarding to education level, the primary participants were participated high with 55 (41.0%), and followed by secondary with 30 (22.4%), university with 20 (14.9%), illiterates with 19 (15.6%), respectively (Table 1).

Table 1
Demographic characteristics of study participants (n = 133)

Variables Categories		Frequence	Percentage %
Age groups	0–10	43	32.1%
	11–21	26	19.4%
	22–32	11	8.2%
	33–43	9	6.7%
	44–54	9	6.7%
	55+	35	26.1%
Gender	Male	48	35.8%
	Female	85	63.4%
Occupation	Student	62	46.3%
	Farmer	26	19.4%
	Unemployed	30	22.4%
	Others job	1	0.7%
Residence	Rural	76	56.7%
	Urban	57	42.5%
Education level	Illiterates	19	14.1%
	Nursery	8	6%
	Primary	55	41.0%
	Secondary	30	22.4%
	University	21	15.6%
Total		133	100%

(Inset Table 1)

Profiles of *E. coli* pathotypes and Salmonella among diarrhea patients at Ruhengeri level 2 Teaching Hospital.

Among *E. coli* strains, the highly prevalent strain was ETEC with 90(67.9%), and followed by EPEC with 30(22.4%), AIEC with 8(6%), EHEC with 3(2.2%) and EAEC with 1(0.7%) respectively. Out of all the tested

isolates, 18 were confirmed as *Salmonella*, corresponding to a prevalence of 13.8% among the study population (Fig. 1).

(Inset Fig. 1)

Antimicrobial susceptibility patterns among *E. coli* pathotypes and *Salmonella*

In *E. coli* isolates, ampicillin and amoxicillin-clavulanic acid exhibit high resistance across most *E. coli* pathotypes especially in EAEC (100%) (Table 3). In contrast, cefotaxime, ceftazidime, meropenem, amikacin, gentamycin, ciprofloxacin and norfloxacin show strong effectiveness, with meropenem 100% sensitivity. Sulfamethoxazole-trimethoprim shows variable resistance, higher in EHEC (66.7%). For *Salmonella* isolates, among the tested antibiotics, meropenem, showed the highest effectiveness with 100% susceptibility, and was followed by Cefepime with 96.9%, equal proportion of susceptibility was observed in Ciprofloxacin, and Norfloxacin (94.7%), cefotaxime and ceftazidime (94.44%), and was followed by sulfamethoxazole-trimethoprim (88.88%). While ampicillin exhibited the highest resistance at 83.3%, followed by moderate resistance to amikacin and gentamycin (22.22%), and lower resistance to amoxicillin-clavulanic acid (16.66%) (Table 2).

Table 2
Antimicrobial susceptibility patterns among *E. coli* pathotypes and *Salmonella*

		<i>E. coli</i> Pathotypes					<i>Salmonella</i>
		ETEC	EPEC	EIEC	EHEC	EAEC	
Ampicillin	R%	82.40%	70%	75%	33.30%	100%	83.30%
	S %	9.90%	10%	0.00%	0%	0%	5.55%
	I %	7.70%	20%	25%	66.7%%	0%	2%
Amoxicillin-clavulanic	R%	7.70%	10%	75%	33.30%	0%	16.66%
	S %	90.10%	90%	12.50%	66.70%	100%	77.77%
	I %	2.20%	0%	12.50%	0%	0%	5.60%
Cefotaxime	R%	12.10%	13.30%	12.50%	66.70%	0%	5.55%
	S %	87.80%	86.70%	87.50%	33.30%	100%	94.44%
	I %	0%	0%	0%	0%	0%	0%
Ceftazidime	R%	13.20%	10%	12.50%	66.70%	0%	5.55%
	S %	86.80%	90%	87.20%	33.30%	100%	94.44%
	I %	0%	0%	0%	0%	0%	0%
Meropenem	R%	0%	0%	0%	0%	0%	0%
	S%	100%	100%	100%	100%	100%	100%
	I %	0%	0%	0%	0%	0%	0%
Amikacin	R%	7.70%	6.70%	0%	0%	0%	22%
	S %	92.30%	93.30%	100%	100%	100%	72.22%
	I %	0%	0%	0%	0%	0%	5.50%
Gentamycin	R%	7.70%	6.70%	0%	0%	0%	22%
	S %	92.30%	93.30%	100%	100%	100%	72.22%
	I %	0%	0%	0%	0%	0%	5.50%
Ciprofloxacin	R%	6.60%	6.70%	0%	0%	0%	5.26%

	S %	93.40%	93.30%	100%	100%	100%	94.70%
	I %	0%	0%	0%	0%	%	0%
	R%	6.60%	3.30%	0%	0%	0%	5.60%
Norfloxacin	S %	93.40%	96.70%	25%	100%	100%	94.40%
	I %	0%	0%	75%	0%	0%	0%
	R%	13.20%	3.30%	0%	66.70%	0%	11.11%
Sulfamethoxazole-trimethoprim	S %	86.80%	96.70%	100%	66.70%	0%	88.88%
	I %	0%	0%	0%	33.30%	100%	0%
	R%	13.20%	3.30%	0%	66.70%	0%	11.11%

Table 3
Genotypic characterization of *E. coli* pathotypes, and *salmonella*

		ESBL resistance genes					
		bla-CTX		Bla-SHV		bla-TEM	
		Present	Absent	Present	Absent	Present	Absent
E. coli	ETEC	6(19.35%	12(38.7%	4(12.09%	12(38.7%	6(19.35%	10(32.25%)
	EHEC	0(0%)	3(9.67%)	3(9.67%)	0(0%)	0(%)	3(9.67%)
	EPEC	1(3.22)	6(19.35%	0(0%)	9(29.03%	0(0%)	9(29.03%)
	EIEC	0(0%)	3(9.67%)	0(0%)	3(9.67%)	0(0%)	3(9.67%)
	EAEC	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Salmonella		3/30%	7/70%	7/70%	3/30%	2/20%	8/80%

(Inset Table 2)

Phenotypic determination of ESBL production among *E. coli* pathotypes and *Salmonella* isolates

Out of 133 *E. coli* isolates, 31(23%) were positive to *Extended spectrum Beta-lactamase* production. EAEC had 1 isolates, and none were (0%) ESBL produces. EHEC had 7 isolates, with1 (14.3%) tested ESBL positive. EIEC had 8 isolates, with 2(25%) ESBL positive, EPEC had 22 isolates, with 4 (18.2%) positive for ESBL, and ETEC had a highest frequency, with 95 isolates and 27(28.4) were ESBL positive. Among 18 *Salmonella* isolates tested for phenotypically ESBL production, 10 were ESBL positive with 55.5% (Fig. 2).

(Inset Fig. 2)

Genotypic characterization of *E. coli* pathotypes, and salmonella isolates

The distribution of ESBL resistance genes among different *E. coli* pathotypes revealed notable variability. ETEC showed the highest overall prevalence, with CTX, SHV, and TEM genes detected in 19.35%, 12.09%, and 19.35% respectively. EPEC exhibited lower resistance, with only CTX detected in 3.22% and no SHV or TEM genes present. EHEC and EIEC showed limited resistance genes, with only SHV detected in 9.67% of EHEC isolates and no ESBL genes identified in EIEC. In *Salmonella* isolates, among the tested isolates, the SHV gene was the most frequent detected, and was found in 7 out of 10 isolates (70%). Both CTX-M, and TEM showed moderate prevalent with 3/30% and 2/20% respectively (Table 3)

(Inset Table 3)

DISCUSSION

This study examined demographic and clinical characteristics of patients presenting with diarrhea symptoms. The most affected age group was children aged 0–10 years, accounting for 32.1% of the sample, followed by older adults above 55 years (26.1%) (Table 1). These findings align with global patterns that recognize young children and older adults as particularly vulnerable to diarrheal illnesses due to their underdeveloped or weakened immune systems [2], [7]. A study by McClelland *et al.* (2022) in Tanzania similarly reported most participants were children under five years (28.5), largely due to immature immunity and environmental exposure, particularly in low-resource settings.

The predominance of females (63.4%) in this study contrasts the findings by Tareke& Takele, (2022) they reported that male-dominant gender distribution in diarrheal cases may be due to women especial in low- and middle-income setting are more frequently involved in house hold task. In contrast, a study by Ahmed *et al.* (2021) in Bangladesh found that male (53.7%) was more likely to present with diarrhea than females (46.3). Similar findings were estimated by the study done in Sub Saharan African Countries from 2014–2021 by Ahmed *et al.* (2024) female participated higher than male, this may be due to increase awareness for health.

Occupational data indicated that students were the most affected group (46.3%), likely due to school environments where person-to-person transmission is higher [21]. The educational background of the participants also suggests a higher burden among those with lower educational attainment. Participants with primary education or none constituted the majority (41.0% and 15.6% respectively), supporting evidence that limited health literacy can increase the risk of diarrheal infections [25].

The result in Fig. 1 identified enterotoxigenic *Escherichia coli* (ETEC) as the most predominant strain among patients with diarrhea symptoms, accounting for 67.9% of the isolates. The high prevalence of ETEC is consistent with global data suggesting that ETEC is a leading cause of acute diarrheal illness,

particularly in developing countries and among young or adult populations [2]. A similar distribution pattern was reported by Lanata & Black. (2018) which found that ETEC was the most commonly isolated strain among children with diarrhea in endemic areas. The same as in Western Ethiopia a study conducted by Mulu *et al.* (2024) showed that ETEC to be the most abundant among *E. coli* pathotypes cause diarrhea in children due to production of toxin with 53.8% and followed by EPEC 12.8%, EAEC with 10.3%, and EHEC (7.7%). Study conducted in Rwanda revealed that ETEC was 12% and the most cause of diarrhea among children's patients [12], this is lower compared to the present study. Diarrhea caused by ETEC is attributed by toxin production, which used by the bacteria to colonize small intestine and by production of heat labile and heat stable enterotoxin [21].

The current study showed that EAEC is at 0.7% which is more different from the report done in upper Egypt by Khairy *et al.* (2020) showed that EAEC to be the most present pathotypes with 47% among all *E. coli* pathotypes. The same different was observed by Montes-yedra *et al.* (2024) in Southwestern Mexico, EPEC (51%) was the most prevalence among children with diarrhea but the present study showed (EPEC) at 22.4%. In 2023, study carried out in Per-Urban of Lusaka showed that EPEC with 45.4% and followed by EAEC (39.3%) [29]. The report done on clinical and environmental samples showed that EHEC (49.5%) was the most prevalent, which is different from current study where, EHEC was lower prevalent compared to other pathotypes with (2.2%). This discrepancy may be due to regional differences in strain circulation, host susceptibility, diagnostic methods, or temporal factors influencing *E. coli* ecology [29].

The current study, the prevalence of *Salmonella* was 13.8% among patients presenting with diarrheal symptoms (Fig. 1). This finding indicates that *Salmonella* species remains a significant bacterial contributor to gastroenteritis. In Ethiopia, the finding reported by Hospital *et al.* (2025) where low prevalence of *salmonella* was reported with 6.3%. However, these results contrast with findings from Msolo *et al.* (2020) who reported a higher prevalence of *Salmonella* (23%) in their investigation among outpatients in South Africa. Increase prevalence of *salmonella* among diarrhea patients underscores the need for improved food safety measures, public education on hygiene practices, and regular microbial surveillance [32].

Regarding antimicrobial susceptibility, *E. coli* isolates in current study exhibited high resistance to ampicillin (82.4% in ETEC), and 75% for EIEC (Table 2). This pattern is consistence with findings from Mulu *et al.* (2024) in Northwestern Ethiopian, where ampicillin resistance among all *E. coli* pathotypes was reported at 74%. Mulu 's study also noted that 84.6% of isolates were MDR, showing up to ten different antibiotics resistance patterns, including resistance to ampicillin. This study showed that Meropenem was 100% effective against all *E. coli* pathotypes, indicating its continued reliability as treatment option for severe cases. Ceftazidime, amikacin and gentamycin were also high effective against all *E. coli* pathotypes except for EHEC with 33% resistance to ceftazidime and Sulfamethoxazole-trimethoprim (66.7%).

Comparative trends were observed in Msolo *et al.* (2020), where *E. coli* isolates were also high susceptible to meropenem, amikacin (95%), and gentamycin (93%), supporting the findings of the current study. However, Msolo's report highlighted a notable resistance to ciprofloxacin (60%), which is different from current study with high susceptibility rate of 93.4%. This result from overuse or misuse of antibiotics in health facilities, leading to localized resistance pressure [16].

In contrast, Ulom *et al.* (2022) from Nigeria reported 28% resistance to meropenem in *E. coli* isolates. Such contradictory results may be attributed to differences in the antibiotics usage policies, self-medication practice, which can increase resistance to even last-line drugs like carbapenem. In Western Mexico, the study carried out by Montes-yedra *et al.* (2024) estimated that ETEC was resisted to meropenem at 25% and 100% to cefazolin. This may be due to antibiotics stewardship challenges.

The antimicrobial susceptibility profile of *Salmonella* isolates in this study highlights a dual pattern of high susceptibility to advanced-generation antibiotics and alarming resistance to commonly used first-line agents. Meropenem was found to be the most effective antibiotic, exhibiting 100% susceptibility, followed by cefepime (96.9%), ciprofloxacin and norfloxacin (94.7%), and cefotaxime (94.4%) (Table 3). These findings reflect the continued effectiveness of carbapenems, third- and fourth-generation cephalosporins, and fluoroquinolones in managing *Salmonella* infections.

These results are consistent with findings from Mahindroo *et al.* (2024) who reported similarly high sensitivity of *Salmonella enterica* to Meropenem and cefepime (100%), cefotaxime 80%, in India, however, it showed low susceptibility to norfloxacin, and ciprofloxacin both with 40% of susceptibility patterns. The efficacy of these agents likely stems from their broad-spectrum activity and limited overuse compared to older antibiotics. The relatively high susceptibility to sulfamethoxazole-trimethoprim (88.9%) in this study also suggests that, despite historical resistance, this drug may still be a viable treatment option in some local contexts.

In contrast, ampicillin exhibited the highest resistance rate at 83.3%, underscoring its declining utility as a front-line treatment for *Salmonella* infections. This is concerning given that ampicillin has historically been a mainstay therapy for enteric pathogens. Similarly, report was observed from Amathole District by Msolo *et al.* (2020). where the high resistance to Ampicillin was 95%. These resistance patterns may be attributed to the widespread and often inappropriate use of antibiotics, especially in community settings without laboratory guidance.

Phenotypic determination for ESBL expression in this study estimated that ETEC exhibited the highest number of ESBL producing isolates and genotypically, carrying both blaTEM and blaCTX-M genes (Fig. 2). Similarly, EIEC and EPEC demonstrated genotypic ESBL positive but very limited detection of the three major genes, suggesting possible involvement of the other resistance mechanisms or genes not screened in this study. Interestingly, these findings differ from those reported by Shahbazi *et al.* (2021) who observed no ESBL resistance genes in ETEC isolates. Their study identified EAEC as the resistance pathotypes with 32% of isolates carrying the TEM gene, and 83.3% phenotypic ESBL expression. These findings reflect the growing challenge posed by ESBL-producing enteric pathogens in clinical settings,

particularly in regions where antibiotic mis-use and poor infection control practices are common. The presence of ESBL genes in EIEC and EHEC, both of which are associated with severe or invasive forms of diarrhea, raises particular concern. The production of ESBL enzymes such as CTX-M, TEM, or SHV renders these strains resistant to a broad range of β -lactam antibiotics, including third-generation cephalosporins etc, often leaving only limited treatment options like carbapenems.

Current study is consistent with a study by Bahgat *et al.* (2024) in Egypt, which reported a high prevalence of ESBL genes in EHEC and ETEC strains, indicating that horizontal gene transfer and clonal expansion of resistant strains are becoming increasingly common. A contradicted finding was observed in the study conducted in Egypt by El-shaer *et al.* (2021) where the most strain with ESBL was in EPEC with 42.7% in diarrhea patients. El-Shaer emphasized that among all *E. coli* pathotypes, the blaTEM gene was the most commonly detected, followed by SHV and CTX-M genes. Different results were observed in the study conducted by Khairy *et al.* (2020) where EAEC showed high prevalent of blaTEM gene with 41.9% but no SHV genes detected among all *E. coli* pathotypes. The differences in ESBL gene prevalence across various *E. coli* pathotypes highlight the need for pathotypes specific surveillance in antimicrobial resistance research.

Among the tested isolates, the blaSHV gene was the most frequently detected, found in 7 out of 10 isolates (70%) (Table 3). The current study, the most ESBL gene detected was blaSHV, this contrasts the findings from recent studies, where blaCTX-M gene was the most dominant ESBL gene. For example, in 2024 clinical study in China CTX-M was the most prevalent with 12.6% of *salmonella enterica* in human isolate [34]. Other study estimated that SHV and TEM were far less frequent among diarrhea patients [37]. Furthermore, the potential for antimicrobial resistance among *Salmonella* strains requires integrated monitoring to guide effective clinical management and antibiotic stewardship.

Limitation

This study had limited sample size and ESBL resistance gene detection, which restricts generalizability of the findings to the wider population. Additionally, only a subset of bacterial isolates was tested for ESBL resistance genes using molecular methods, which may have led to underreporting or an incomplete understanding of the actual gene distribution.

Conclusion

This study identified ETEC as the most dominant *E. coli* pathotypes with 67.9% among diarrhea patients followed by EPEC (22.4%). *Salmonella enterica* was also significantly present in 13.8%. Alarming, the majority of isolates high resistance to commonly used antibiotics including ampicillin and sulfamethoxazole/trimethoprim while meropenem remain against most isolates. On genetic level, SHV was the most prevalent ESBL gene, followed by CTX-M, and TEM. These findings demonstrate the urgency of implementing strong antimicrobial resistance surveillance system, as well as localized guideline for treatment of diarrhea infections.

Abbreviations

AMR

Antimicrobial resistance

CLSI

Clinical and Laboratory Standard Institute

ESBL

Extended Spectrum Beta Lactamase

SHV

Sulfhydryl Variable (a type of ESBL gene)

TEM

Temoneira (a type of ESBL gene, named after a Greek patient)

CTX-M

Cefotaxime-Munich (ESBL gene)

DAEC

Diffuse Adherence *Escherichia coli*

DEC

Diarrheagenic *Escherichia coli*

DNA

Deoxyribonucleic acid

EAEC

Enteropathogenic *Escherichia coli*

EHEC

Enterohaemorrhagic *Escherichia coli*

EIEC

Enteroinvasive *Escherichia coli*

EMB

Eosin Methylene Blue Agar

EPEC

Enteropathogenic *Escherichia coli*

ETEC

Enterotoxigenic *Escherichia coli*

Bp

Base Paire

IMViC

Indole, Methyl Red, Voges-Proskauer, Citrate (biochemical tests)

MDR

Multi-drug Resistance

NLF

Non-Lactose Fermenting

PCR

Polymerase Chain Reaction

CLSI

Clinical and Laboratory Standards Institute

RLTTH

Ruhengeri Level 2 Teaching Hospital

WHO

World Health Organization

XLD

Xylose Lysine Deoxycholate Agar

SPSS

Statistical Package for Social Sciences

Declarations

Ethics approval and consent to participation

Ethical approval was waived by the Institutional Review Board (IRB) of INES-Ruhengeri University in collaboration with Ruhengeri Level 2 Teaching Hospital. The samples were collected from diarrheic patients after obtaining informed consent from participants or their legal guardians. Participant confidentiality and privacy were strictly maintained throughout the study. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, and all methods were performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

This study does not contain any individual person's data, images, or identifiable information requiring consent for publication. All data presented are anonymized and reported in aggregate form to ensure participant confidentiality.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to ethical and confidentiality restrictions but are available from the corresponding author on the reasonable request.

Competing interests

The authors declare that there are no conflicts of interest related to this study.

Fundings

This research received no specific grant from any funding agency.

Authors contributions

"YI research Project idea, conceptualization, methodology design, data collection, laboratory investigation, drafting of the manuscript. CM supervision of laboratory work, validation of results, critical manuscript revision. LM statistical analysis. EB data curation, laboratory work support. HM Sample collection. PM safety precaution. BN technical support in Molecular biology. MJ molecular biology workload support. TH molecular biology results interpretation. CY overall study supervision, and final manuscript review. All authors read and approved the final manuscript."

Acknowledgments

The authors would like to express their sincere gratitude to Gregory Hemphill for his generous financial support, which make this study possible. The authors would like to express their sincere gratitude to the staff of INES Ruhengeri university and Ruhengeri Level 2 Teaching Hospital staff for their valuable support and cooperation during the study period. We also extend our heartfelt thanks to all the study participants for their willingness to take part in this research. Their contribution was essential to the successful completion of the study.

Clinical trial number

Clinical trial number: **not applicable**

References

1. Mokomane M, Kasvosve I, de Melo E, Pernica JM, Goldfarb DM. The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. *Ther Adv Infect Dis*. 2018;5:29–43.
2. Tareke AA, Enyew EB, Takele BA. Pooled prevalence and associated factors of diarrhea among under-five years children in East Africa: A multilevel logistic regression analysis. *PLoS ONE*. 2022;17:1–16.
3. Zhao WZ, Wang JY, Zhang MN, Wu SN, Dai WJ, Yang XZ, Wang HG. Global burden of diarrhea disease in the older adult and its attributable risk factors from 1990 to 2021: a comprehensive analysis from the global burden of disease study 2021. *Front Public Health*. 2025;13:1541492.
4. Magzuop RFA, Hammad MA, Idris AAA. The common aerobic bacteria causing diarrhea in children in Omdurman hospital-Sudan. *Microbes Infect Dis*. 2024;5:242–8.
5. Alhadlaq MA, Aljurayyad OI, Almansour A, Al-Akeel SI, Alzahrani KO, Alsalman SA, et al. Overview of pathogenic *Escherichia coli*, with a focus on Shiga toxin-producing serotypes, global outbreaks (1982–2024) and food safety criteria. *Gut Pathog*. 2024;16:57.

6. Msolo L, Iweriebor BC, Okoh AI. Pervasiveness of diarrheagenic *E. coli* pathotypes and *Salmonella* species among gastroenteritis patients in some selected pastoral hinterlands of the Amathole district municipality, Eastern Cape, South Africa. *Hum Microbiome J.* 2020;17:100074.
7. Li Y, Xia S, Jiang X, Feng C, Gong S, Ma J, et al. Gut Microbiota and Diarrhea: An Updated Review. *Front Cell Infect Microbiol.* 2021;11:1–8.
8. Thystrup C, Majowicz SE, Kitila DB, Desta BN, Fayemi OE, Ayolabi CI, et al. Etiology-specific incidence and mortality of diarrheal diseases in the African region: a systematic review and meta-analysis. *BMC Public Health.* 2024;24:1864.
9. Diouf K, Tabatabai P, Rudolph J, Marx M. Diarrhoea prevalence in children under five years of age in rural Burundi: an assessment of social and behavioural factors at the household level. *Glob Health Action.* 2014;7:2489510.
10. Veraldi S, Benzecry V, Faraci AG, Nazzaro G. Allergic contact dermatitis caused by paromomycin. *Contact Dermat.* 2019;81:393–4.
11. Kaneza D, Kidane AA, Kubahoniyesu T. Prevalence and associated factors of diarrhea among children under five years of age in the Western Province of Rwanda: analysis of the Rwanda Demographic and Health Survey (RDHS) 2019–2020. *PAMJ-One Health.* 2025;16:7.
12. Munyemana JB, Kabayiza JC, Nilsson S, Andersson ME, Lindh M. Shigella and ETEC have replaced rotavirus as main causes of childhood diarrhea in Rwanda after ten years of rotavirus vaccination. *J Infect Dis.* 2024;230:1176–80.
13. Wallace MJ, Fishbein SRS, Dantas G. Antimicrobial resistance in enteric bacteria: current state and next-generation solutions. *Gut Microbes.* 2020;12:1799654.
14. Bezabih YM, Sabiiti W, Alamneh E, Bezabih A, Peterson GM, Bezabhe WM, et al. The global prevalence and trend of human intestinal carriage of ESBL-producing *Escherichia coli* in the community. *J Antimicrob Chemother.* 2021;76:22–9.
15. Ramos S, Silva V, de Lurdes Enes Dapkevicius M, Caniça M, Tejedor-Junco MT, Igrejas G, et al. *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: Health implications of extended spectrum β -lactamase (ESBL) production. *Animals.* 2020;10:1–15.
16. Muriuki C, Kairigo P, Home P, Ngumba E, Raude J, Gachanja A, et al. Mass loading, distribution, and removal of antibiotics and antiretroviral drugs in selected wastewater treatment plants in Kenya. *Sci Total Environ.* 2020;743:140655.
17. Mahindroo J, Thanh DP, Kaur H, Nguyen THT, Carey ME, Verma R, et al. The genomic diversity and antimicrobial resistance of Non-typhoidal *Salmonella* in humans and food animals in Northern India. *One Health.* 2024;19:100892.
18. Ibekwe A, Durso L, Ducey TF, Oladeinde A, Jackson CR, Frye JG, et al. Diversity of plasmids and genes encoding resistance to extended-spectrum β -lactamase in *Escherichia coli* from different animal sources. *Microorganisms.* 2021;9:1–16.
19. Kaderabkova N, Bharathwaj M, Furniss RCD, Gonzalez D, Palmer T, Mavridou DAI. The biogenesis of β -lactamase enzymes. *Microbiology.* 2022;168:001217.

20. Lascols C, Lascols C, Molecular G. Global Molecular Characterization of Extended-Spectrum β -lactamases and carbapenemases in Enterobacterales To cite this version. HAL Id; 2024. pp. tel-04738725.
21. Msolo L, Iweriebor BC, Okoh AI. Antimicrobial resistance profiles of diarrheagenic e. Coli (dec) and salmonella species recovered from diarrheal patients in selected rural communities of the amathole district municipality, Eastern cape province, South africa. *Infect Drug Resist*. 2020;13:4615–26.
22. McClelland PH, Kenney CT, Palacardo F, Roberts NLS, Luhende N, Chua J, et al. Improved Water and Waste Management Practices Reduce Diarrhea Risk in Children under Age Five in Rural Tanzania: A Community-Based: Cross-Sectional Analysis. *Int J Environ Res Public Health*. 2022;19:1–18.
23. Ahmed T, Chisti MJ, Rahman MW, Alam T, Ahmed D, Parvin I, et al. Effect of 3 Days of Oral Azithromycin on Young Children with Acute Diarrhea in Low-Resource Settings: A Randomized Clinical Trial. *JAMA Netw Open*. 2021;4:e2136726.
24. Ahmed KY, Dadi AF, Kibret GD, Bizuayehu HM, Hassen TA, Amsalu E, et al. Population modifiable risk factors associated with under-5 acute respiratory tract infections and diarrhoea in 25 countries in sub-Saharan Africa (2014–2021): an analysis of data from demographic and health surveys. *eClinicalMedicine*. 2024;68:102444.
25. Lanata CF, Black RE. Estimating the true burden of an enteric pathogen: enterotoxigenic Escherichia coli and Shigella spp. *Lancet Infect Dis*. 2018;18:1165–6.
26. Mulu BM, Belete MA, Demlie TB, Tassew H, Tessema TS. Characteristics of Pathogenic Escherichia coli Associated with Diarrhea in Children under Five Years in Northwestern Ethiopia. *Trop Med Infect Dis*. 2024;9:65.
27. Khairy RMM, Fathy ZA, Mahrous DM, Mohamed ES, Abdelrahim SS. Prevalence, phylogeny, and antimicrobial resistance of Escherichia coli pathotypes isolated from children less than 5 years old with community acquired- diarrhea in Upper Egypt. *BMC Infect Dis*. 2020;20:1–9.
28. Tapia-Pastrana G, Rojas-Bautista M, Hernández-Pérez P, Santiago-Martínez O, Gómez-Rodríguez LC, Terrazas-Luna VM, et al. Virulence genes, antimicrobial resistance profile, phylotyping and pathotyping of diarrheagenic Escherichia coli isolated from children in Southwest Mexico. *PLoS ONE*. 2024;19:e0300304.
29. Mwape K, Bosomprah S, Chibesa K, Silwamba S, Luchen CC, Sukwa N, et al. Prevalence of Diarrhoeagenic Escherichia coli among Children Aged between 0–36 Months in Peri-Urban Areas of Lusaka. *Microorganisms*. 2023;11:1–13.
30. Dade Y, Kannaiyan M, Dedecha W, Daka D, Husen O, Gemechu T. Prevalence, antimicrobial susceptibility pattern and associated factors of Salmonella and Shigella among under five children with diarrhea attending Bule Hora University Teaching Hospital, Bule Hora, West Guji, Ethiopia. *BMC Infect Dis*. 2025;25:571.
31. Abera K, Anticho TL, Ali MM. Salmonella and Shigella and antimicrobial susceptibility profiles among adult patients with complaints of diarrhea at Hawassa comprehensive specialized Hospital, Hawassa, Ethiopia. *SAGE Open Med*. 2021;9:20503121211000911.

32. Azanaw J, Malede A, Yalew HF, Worede EA. Determinants of diarrhoeal diseases among under-five children in Africa (2013–2023): a comprehensive systematic review highlighting geographic variances, socioeconomic influences, and environmental factors. BMC Public Health. 2024;24:2399.
33. Ulom UI, Ibeneme EO, Eyo AAO, Asuquo AE, Utsalo SJ. Drug resistance profiles of Gram-negative bacilli isolated from clinical samples in Cross River State, Nigeria. Calabar J Heal Sci. 2022;6:92–9.
34. Shahbazi G, Ahangarzadeh M, Nikkhahi F, Gholizadeh P. Characteristics of diarrheagenic Escherichia coli pathotypes among children under the age of 10 years with acute diarrhea. Gene Rep. 2021;25:101318.
35. Bahgat OT, Rizk DE, Kenawy HI, Barwa R. Characterization of non-O157 enterohemorrhagic Escherichia coli isolated from different sources in Egypt. BMC Microbiol. 2024;24:488.
36. El-shaer S, Id SHA, rhman, Barwa R, Hassan R. Genetic characterization of extended- spectrum β - Lactamase- and carbapenemase- producing Escherichia coli isolated from Egyptian hospitals and environments. PLoS ONE. 2021;19:e0303719.
37. Zhang S, Yang J, Abbas M, Yang Q, Li Q, Liu M, et al. Threats across boundaries: the spread of ESBL-positive Enterobacteriaceae bacteria and its challenge to the one health concept. Front Microbiol. 2025;16:1496716.

Figures

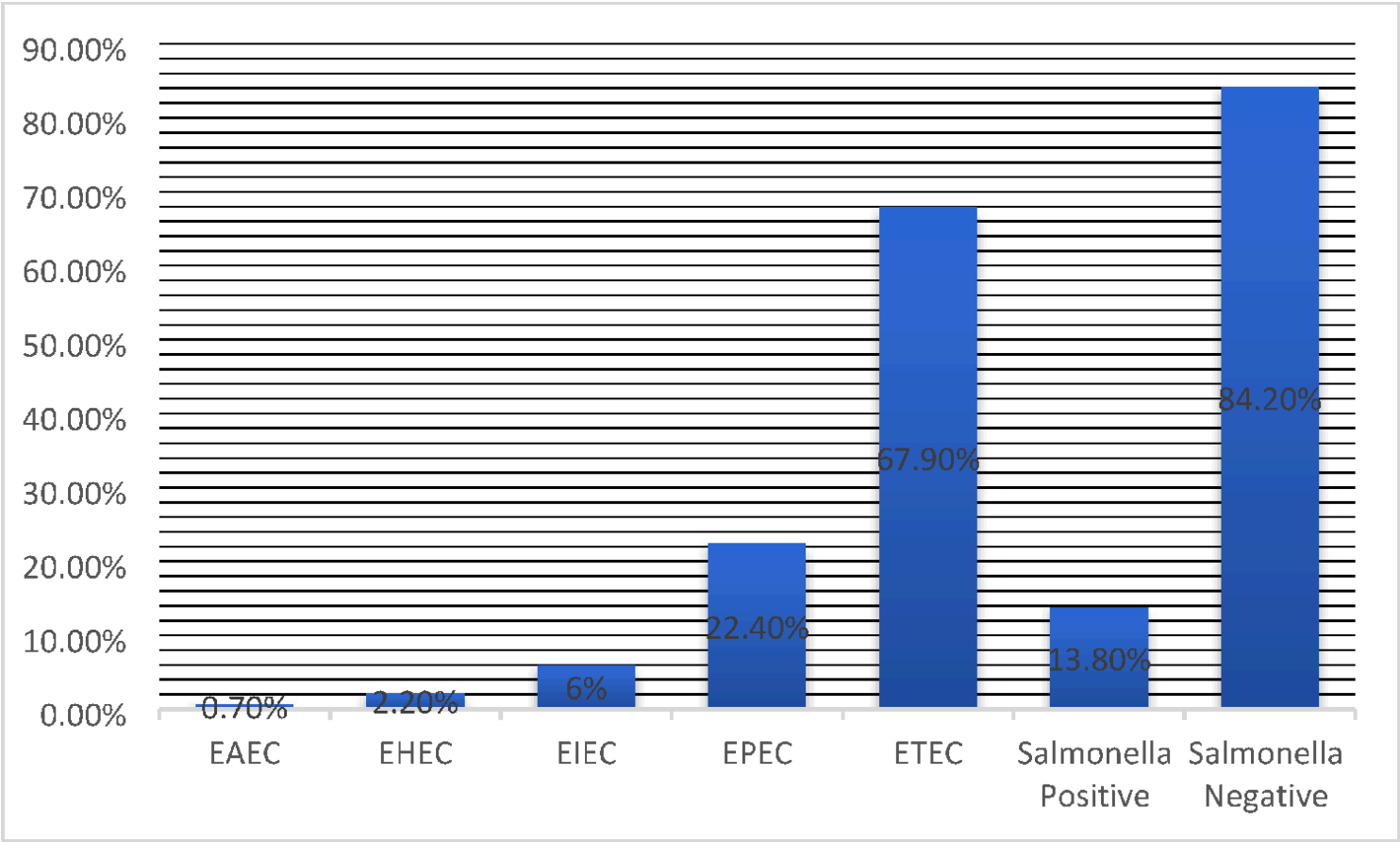


Figure 1

Profiles of *E. coli* and *Salmonella* pathotypes among diarrhea patients at Ruhengeri level 2 Teaching Hospital.

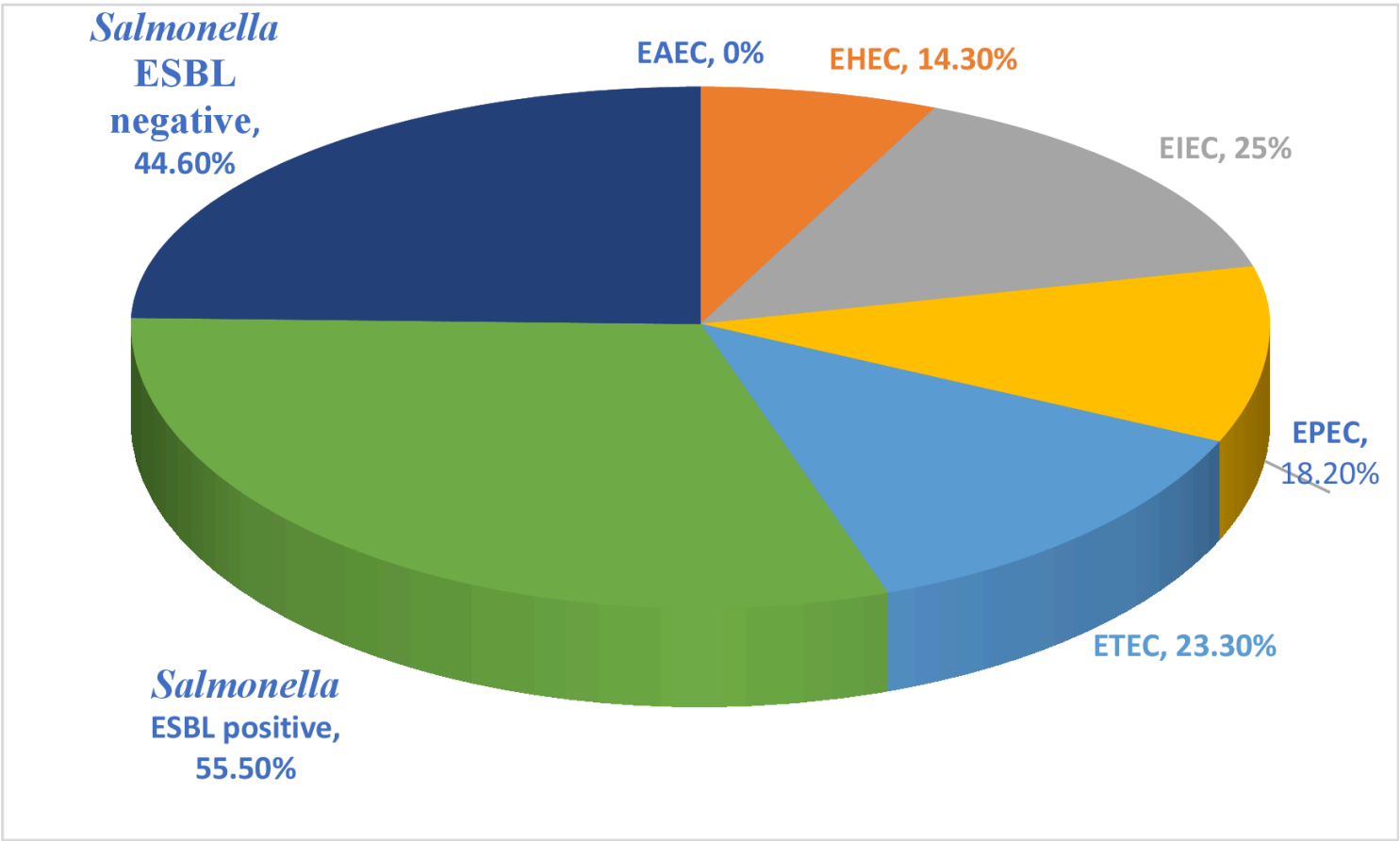


Figure 2

Phenotypic determination of ESBL production among *E. coli* pathotypes and *Salmonella* isolates.