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Extended-Spectrum β-Lactamases-Producing *Enterobacteria* and Antimicrobial Resistance Pattern among HIV/AIDS Patients in the University of Gondar Specialized Hospital, Ethiopia

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Abstract

There is growing evidence indicating that drug-resistant bacteria notably Extended spectrum β-lactamases producing *Enterobacteria* are greatly implicated in immunocompromised groups namely HIV-infected patients. Little is known about the burden of ESPL-E in places where HIV infection is rampant. Therefore, the study was aimed to assess the prevalence, antimicrobial resistance pattern of ESPL-E among HIV/AIDS patients. A cross-sectional study was conducted among HIV/AIDS patients seeking Antiretroviral Treatment (ART) service at the University of Gondar Hospital from February–May, 2017. The pretested and -structured questionnaire was used to collect data on sociodemographic and clinical-related factors. Clean catch midstream urine samples were collected and cultured in line with standard procedures. The drug susceptibility testing was performed by Kirby Bauer disc diffusion method. Extended-spectrum β-lactamase (ESBL) detection was performed using a double-disc synergy test and combined disc methods. Data entry and analysis were performed using SPSS version 20. Among a total of 387 HIV/AIDS patients, 42 (10.9%) *Enterobacteria* uropathogens were identified. Among these isolates, nine (21.4%) were ESBL producers. The highest prevalence of ESBL production was *Escherichia coli* (44.4%) followed by *Klebsiella pneumoniae* (22.2%) and *Enterobacter* spp. (22.2%). Higher drug resistance rates were observed among ESBL-producing isolates compared to ESBL-nonproducing isolates. Amox-clavulanic (100%), ampicillin (95%), cotrimoxazole (74%), cefotaxime (88.9%), and ceftazidime (88.9%) had high resistance rates to Extended spectrum β-lactamases producing *Enterobacteria*. The overall prevalence of multidrug resistance of all isolates was 92.9%, and all the ESBL isolates were multidrug resistant. Therefore, antimicrobial stewardship programs needed to be promoted for the rational use of drugs especially in the management of HIV/AIDS patients.

Keywords: Antimicrobial resistance; *Enterobacteria*; Extended-spectrum β-lactamases.

1. INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) are a plasmid-mediated heterogeneous group of transferable β-lactamase enzymes that hydrolyze penicillins, cephalosporins, aztreonam, and other antibiotics but not cephamycins and carbapenems. It also blocks *in vitro* by β-lactamase inhibitors such as clavulanate, tazobactam, and sulbactam [1-3].

Infections caused by ESBL-producing bacteria often involve immune-compromised patients, making it difficult to eradicate these organisms in high-risk wards, such as intensive care units [4, 5]. Serious bacterial infections have emerged as an important cause of morbidity and mortality in individuals with HIV with case-fatality rates of up to 32% especially in patients with low CD_4 count. Individuals with HIV infection are more susceptible and have increased rates of morbidity and mortality to bacterial infections because of defects in both cell-mediated and humoral immunity that frequently lead to multiple treatments.

This situation ultimately facilitates the emergence of drug-resistant isolates via selective pressure, as the survived strains are free to outnumber their population making the treatment ineffective. Moreover, many HIV patients are given primary cotrimoxazole prophylaxis that might increase the risk of antibiotic resistance in a variety of bacterial pathogens in the high-risk population. As a result, the management of bacterial infections in HIV patients is important for preventing further emergence of drug resistance [6].

ESBL strains have been associated with resistance to other non-β-lactam antibiotics such as aminoglycosides and chloramphenicol. Another challenging property related to ESBL strains is that they might show a false sensitivity *in vitro* testing that

leads to inappropriate antibiotic selection in infections caused by these organisms resulting in treatment failures, poor clinical outcomes, prolonged hospital stay, delayed initiation of appropriate antibacterial therapy, increased morbidity, mortality, and healthcare costs [7, 8].

To date, little is known about the overall epidemiology of ESBL-E, especially in immunocompromised individuals. Therefore, this study was aimed to figure out the burden and antimicrobial resistance pattern of ESBL-E isolates among HIV/AIDS patients.

2. METHOD(S)

A hospital-based cross-sectional study was conducted among HIV/AIDS patients from February to May, 2017 at the University of Gondar Hospital, Northwest Ethiopia. The hospital provides services for at least 5 million population living in and around Gondar including Antiretroviral treatment services for HIV/AIDS patients. Currently, the ART clinic provides services for 5,094 ART patients and 141 pre-ART patients.

The study population was the HIV/AIDS patients seeking ART services during the study period in the hospital ART clinic. Patients who had regular ART follow-up were included irrespective of sick from urinary tract infection (UTI), history of UTI and *Escherichia coli* colonization. Those patients with a history of antibiotic use for the past 7 days from data collection were excluded in the study. A total of 387 study participants were included in this study using a systematic random sampling technique.

A midstream clean catch urine sample was collected from each participant. The standard microbiological techniques were used for isolation, identification, and antimicrobial susceptibility testing of bacterial isolates based on Clinical Laboratory Standards Institute (CLSI) guideline [9]. The following antibiotics discs were used in the sensitivity test; cefuroxime (30 μg), cefixime (30 μg), cefotaxime (30 μg), cefpodoxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), nitrofurantoin (100 μg), cotrimoxazole (25 μg), amox-clavulanate (20/10 μg), amikacin (30 μg), and ampicillin (10 μg).

The screening test for ESBL detection was done according to the CLSI guidelines. Isolates showing inhibition zone size below the CLSI-stated breakpoints were considered a potential ESBL producer: cefpodoxime ≤ 22 mm, ceftazidime ≤ 22 mm, cefotaxime ≤ 27 mm, and ceftriaxone ≤ 25 mm [9-11].

Extended spectrum β-lactamase detection was carried out by double-disc synergy tests (DDST) using third-generation cephalosporins and modified double-disc synergy test (MDDST) using cefepime along with the third-generation cephalosporins. A negative DDST or MDDST was confirmed with a combined disc test method.

The double-disc synergy test is used as a primary screening method to identify the ESBL-producing organisms. Isolates that were screened and found positive for ESBL production may be negative for confirmatory tests using DDST due to the coproduction of other β-lactamases like AMPC β-lactamases. In such cases, the modified double-disc synergy test was used with cefepime antibiotics for inhibiting the effect of other β-lactamases (AMPC lactamase) [12, 13].

The reliability of the study findings was guaranteed by implementing quality control (QC) measures throughout the whole process of the laboratory work. Preanalytical, analytical, and postanalytical stages of quality assurance and standard operating procedures (SOPs) were strictly followed. Sterility of culture media was checked by incubating 5% of the batch at 35-37°C overnight and was evaluated for possible contamination. The standard reference bacteria strains of *Klebsiella pneumonia* (ATCC® 700603) were used as positive control and *E. coli* (ATCC® 25922) was used as a negative control of the ESBL detection test [10].

Data were entered and analyzed using SPSS version 20 software. Findings were summarized and tabulated. The statistically significant association was measured by using the Chi-square test, and p-value <0.05 was considered statistically significant.

Ethical approval and official permission were obtained from the University of Gondar, School of Biomedical and Laboratory Sciences, ethical review committee. Written informed consent was obtained from study participants, guardians, or caretakers of children after explaining the purpose and objective of the study. Any patient, who was unwilling to participate in the study, was not forced to participate. They were informed that all data and samples obtained from them were kept confidential by using codes instead of any personal identifiers and is meant only for the study. Laboratory results from the study participants were communicated to their physicians for appropriate treatment or management.

3. RESULTS

3.1. Sociodemographic Characteristics

A total of 387 HIV/AIDS patients were enrolled in the study. The majority of study participants were females (273 [71.1 %]). The mean (SD) age of the study participants was 37.9 ± 9.7 years. Most of the respondents were in the age group of 30-40 years, 159 (41.1%). Besides, 358 (92.5%) of the participants were urban residents, and 210 (54.3%) were also participants who had an educational level of primary school.

3.2. Distribution of *Enterobacteria* in Patient Characteristics

A total of 387 urine samples were collected from HIV/AIDS patients and analyzed accordingly. Among these, 42 (10.9%, 95% CI; 7.8-14.3) patients had a positive culture for *Enterobacteria* result with a single nonduplicate isolate. The culture positivity rate in patients with a history of prior antibiotic use was significantly higher than those who were not (57.1% versus 42.9%, $p < 0.001$).

Similarly, a significant high bacterial isolation rate was observed in patients with a history of antibiotic use without prescription and surgery for the past 6 months (Table 1).

The most common isolated organisms were *E. coli* (29[69%]), followed by *Enterobacter* spp. (6[14.3%]) and *K. pneumoniae* (4[9.5%]). Most importantly, among the isolated *Enterobacteria*, 9 (21.4%, 95% CI: 11.9-31) isolates were found to be ESBL producers with the highest prevalence of *E. coli* (4[44.4%]) followed by *K. pneumoniae* (2[22.2%]), *Enterobacter* Spp. (2[22.2%]) (Table 2).

Table 1: Distribution of *Enterobacteria* per patient characteristics at the University of Gondar Hospital, February–May, 2017.

Variables		Culture positivity (*Enterobacteria*)		
		Negative *N* (%)	Positive *N* (%)	*p*-Value
Gender	Male	96 (85.7)	16 (14.3)	0.166
	Female	249 (90.5)	26 (9.5)	
Age	<30	94 (92.2)	8 (7.8)	0.158
	30-40	136 (85.5)	23 (14.5)	
	>40	115 (91.3)	11 (8.7)	
Occupation	Unemployed	12 (100)	0	0.805
	Daily laborer	19 (90.5)	2 (9.5)	
	House wife	126 (87.5)	18 (12.5)	
	Merchant	82 (88.2)	11 (11.8)	
	Farmer	17 (85)	3 (15)	
	Employed	89 (91.8)	8 (8.2)	
Educational status	Primary and below	187 (89)	23 (11)	0.480
	Secondary school	95 (88)	13 (12)	
	College/University	63 (91.3)	6 (8.7)	
Previous hospitalization for the past 12 months	No	328 (91.9)	29 (8.1)	
	Yes	17 (56.7)	13 (43.3)	<0.001
Previous UTI history	No	283 (90.4)	30 (9.6)	0.099
	Yes	62 (83.8)	12 (16.2)	
Previous antibiotic history	No	287 (94.1)	18 (5.9)	<0.001
	Yes	58 (70.7)	24 (29.3)	
Antibiotic use without prescription	No	330 (90.2)	36 (9.8)	0.007
	Yes	15 (71.4)	6 (28.6)	
Surgery history in the last 6 months	No	329 (90.1)	36 (9.9)	0.011
	Yes	16 (72.7)	6 (27.3)	
Cotrimoxazole prophylaxis	No	47 (85.5)	8 (14.5)	0.342
	Yes	298 (89.8)	34 (10.2)	
Adherence of cotrimoxazole	Not completed	68 (93.2)	5 (6.8)	0.279
	Completed	230 (88.8)	29 (11.2)	
ART status	Pre-ART	16 (80)	4 (20)	0.177
	On ART	329 (89.6)	38 (10.4)	
CD4 count	≤499	191 (88.4)	25 (11.6)	0.608
	≥500	154 (90.1)	17 (9.9)	

Table 2: Extended spectrum β-lactamases producing *Enterobacteria* and non-Extended spectrum β-lactamases producing *Enterobacteria* isolates from HIV/AIDS patients, University of Gondar Hospital, February–May, 2017.

ESBL enzyme production	Bacterial isolates					
	E. coli N (%)	*K. pneumonia N* (%)	*Enterobacter* spp. *N* (%)	*K. ozanae N* (%)	*K. oxytoca N* (%)	Total *N* (%)
Producer	4 (44.4)	2 (22.2)	2 (22.2)	1 (11.2)	0	9 (100)
Nonproducer	25 (75.8)	2 (6.1)	4 (12.1)	1 (3)	1 (3)	33 (100)
Total	29 (69)	4 (9.5)	6 (14.3)	2 (4.8)	1 (2.4)	42 (100)

3.3. Antimicrobial Resistance Pattern of Isolated Organisms

Among the tested antibiotics, all isolates were found to be resistant to amox-clavulanic acid; nearly 95% and 74% of the isolates were also resistant to ampicillin and cotrimoxazole, respectively. More than 75% of *K. pneumoniae* isolates possess a resistance of cefotaxime, ceftazidime, cotrimoxazole, ampicillin, and amox-clavulanic acid. About 29% of ciprofloxacin resistance was observed among the isolates. Specifically, *E. coli, Enterobacter* spp., and *K. pneumoniae* isolates accounted for 8 (27.6%), 2 (33.3%), and 2 (50%), respectively (Table 3). Among the total isolates, 39 (92.9%; 95% CI: 88.1-97.6%) of the isolates showed resistance to three and more classes of antibiotics and which were multidrug-resistant (MDR) organisms. Only three (7.1%) of the isolates were found to be non-MDR strains (resistance either for only one or two classes of drugs). Briefly, 6 (100%) of *Enterobacter* spp., 4 (100%) of *K. pneumoniae,* 2 (100%) of *K. ozanae,* 1 (100%) of *K. oxytoca*, and 26 (89.7%) of *E. coli* isolates were MDR isolates among all isolates.

3.4. Multidrug Resistance Pattern

Interestingly, 39 (92.9%; 95% CI: 88.1-97.6%) of isolates showed resistance to three and more classes of antibiotics that were MDR organisms. Only three (7.1%) of the isolates were found to be non-MDR strains (resistance either for only one or two classes of drugs). Briefly, 6 (100%) of *Enterobacter* spp., 4 (100%) of *K. pneumoniae,* 2 (100%) of *K. ozanae,* 1 (100%) of *K. oxytoca*, and 26 (89.7%) of *E. coli* isolates were MDR isolates among all isolates (Table 4).

Moreover, the total resistance pattern of Extended spectrum β-lactamases producing *Enterobacteria* isolates is presented in Figure 1. All spectrum β-lactamases (ESBL)-producing isolates were 100% resistant to ampicillin and amox-clavulanic acid and 88.9% resistance to cefotaxime and ceftazidime, but the least resistance was observed for both ceftriaxone and cefpodoxime. All Extended spectrum β-lactamases producing *Enterobacteria* isolates were MDR for tested antimicrobials, and 30 (90.9%) of non-Extended spectrum β-lactamases producing *Enterobacteria* isolates were MDR. Antibiotic resistance rate of ESPL-E isolates was significantly higher than non-Extended spectrum β-lactamases producing *Enterobacteria* strains for some of tested antimicrobials like cefuroxime (22.2% versus 3%; $p=0.048$), ceftazidime (88.9% versus 21.2%; $p<0.001$), cefepime (22.2% versus 3%; $p=0.048$), and amikacin (22.2% versus 0%; $p=0.006$). However, significant variation was not observed for the following antibiotics; cefixime, cefotaxime, ceftriaxone, cefpodoxime, ciprofloxacin, cotrimoxazole, ampicillin, amox-clavulanic acid, and nitrofurantoin.

4. DISCUSSION

The overall prevalence of Extended spectrum β-lactamases producing *Enterobacteria* infection was found to be 21.4%, which is comparable with reports indicated in Saudi Arabia (22%) [14]. However, the prevalence noted in this study was significantly higher compared with that documented in studies from the United States (8.6%) [15] and the United Kingdom (1%) [16]. On the other hand, it was lower than reports from different African countries such as Ghana (49.3%) [17] and Uganda (62%) [18].

Table 3: Antibiotic resistance of isolates from HIV/AIDS patients, University of Gondar hospital, February–May 2017.

Drugs	Bacterial isolates					
	E. coli	*Enterobacter* spp.	*K. pneumoniae*	*K. ozanae*	*K. oxytoca*	Total
CRX	2 (6.9)	0	1 (25)	0	0	3 (7.1)
CXM	3 (10.3)	1 (16.7)	1 (25)	0	0	4 (9.5)
CTX	18 62.1)	3 (50)	3 (75)	2 (100)	0	26 (61.9)
CAZ	9 (31)	3 (50)	3 (75)	0	0	15 (35.7)
CTR	0	0	0	1 (50)	0	1 (2.4)
CPD	0	1 (16.7)	1 (25)	0	0	2 (4.8)
CFP	1 (3.4)	1 (16.7)	1 (25)	0	0	3 (7.1)
CPR	8 (27.6)	2 (33.3)	2 (50)	0	0	12 (28.6)
COT	19 65.5)	5 (83.3)	4 (100)	2 (100)	1 (100)	31 (73.8)
AMP	27 (93.1)	6 (100)	4 (100)	2 (100)	1 (100)	40 (95.2)
AMC	29 (100)	6 (100)	4 (100)	2 (100)	1 (100)	42 (100)
NIT	0	0	0	0	0	0
AMK	0	0	1 (25)	1 (50)	1 (100)	3 (7.1)

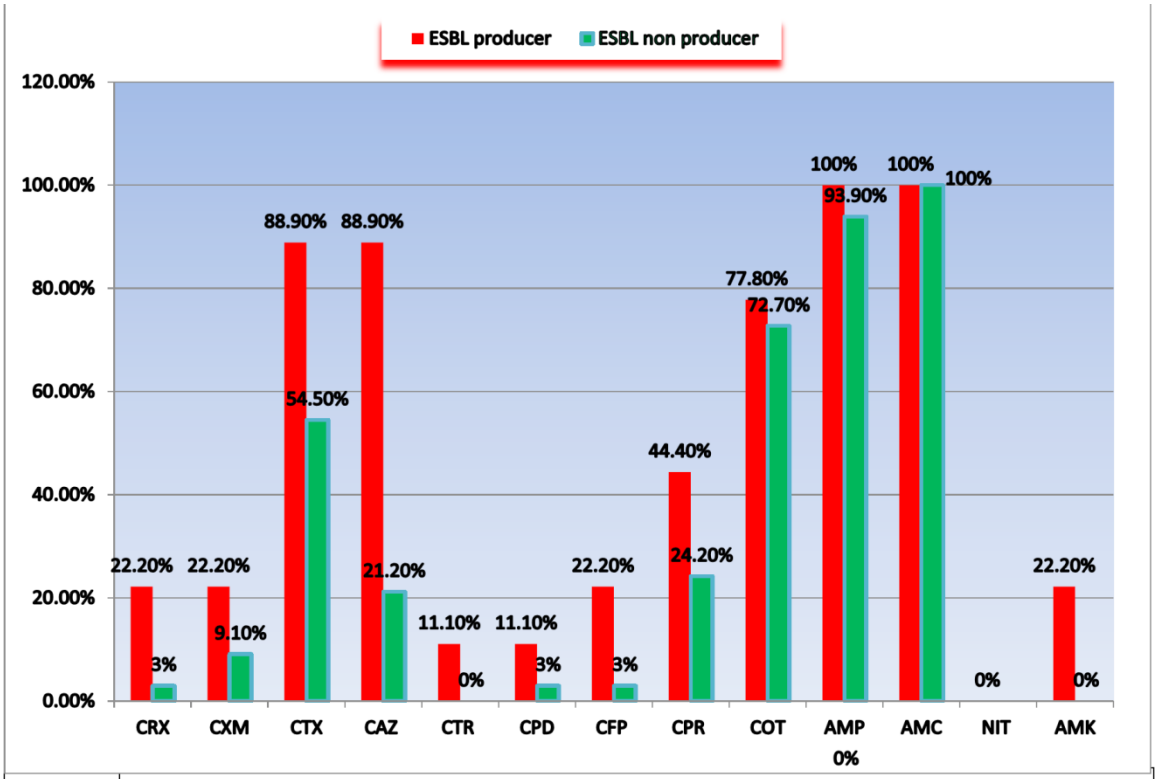
Note: Data are in number (%) unless and otherwise indicated. CRX: cefuroxime, CXM: cefexime, CTX: cefotaxime, CAZ: ceftazidime, CTR: ceftriaxone, CPD: cefpodoxime, CFP: cefepime, CPR: ciprofloxacillin, COT: cotrimoxazole, AMP: ampicillin, AMC: amox-clavulanic acid, NIT: nitrofurantoin, AMK: amikacin.

Table 4: Multidrug resistance pattern of Enterobacteria among HIV/AIDS patients at the University of Gondar Hospital, February–May 2017.

Bacterial isolates	Degree of antibiotic resistance of isolates					
	R2	R3	R4	R5	R6	MDR (≥R_3)
E. coli (*N* = 29)	3 (10.3)	5 (17.2)	10 (34.5)	8 (27.6)	3 (10.3)	26 (89.7)
K. pneumoniae (*N* = 4)	0	0	1 (25)	2 (50)	1 (25)	4 (100)
Enterobacter spp. (*N* = 6)	0	1 (16.7)	3 (50)	0	2 (33.3)	6 (100)
K. ozanae (*N* = 2)	0	0	1 (50)	0	1 (50)	2 (100)
K. oxytoca (*N* = 1)	0	1 (100)	0	0	0	1 (100)
Total (*N* = 42)	3 (7.14)	7 (16.67)	15 (35.71)	10 (23.81)	7 (16.67)	39 (92.86)

Note: MDR: multidrug resistance (R ≥ 3 classes); R2: resistance of isolates for one and two drugs; R3, R4, and R5: resistance of isolates for three, four, and five drugs; R6: resistance of isolates for six and above drugs.

Figure 1: Antibiotic resistance rate of Extended spectrum β-lactamases producing *Enterobacteria* isolates in comparison with non-ESPL-E isolates among study participants at the University of Gondar Hospital, February–May, 2017.



	Antibiotics												
	CRX	CXM	CTX	CAZ	CTR	CPD	CFP	CPR	COT	AMP	AMC	NIT	AMK
p value [a]	**0.048**	0.281	0.060	**<0.001**	0.053	0.313	**0.048**	0.453	0.760	0.449	-	-	**0.006**

The variation might be due to the fact that difference in sampling population, the policy of antibiotics prescription, and socio-cultural and economic factors.

In Ethiopia, there is still limited evidence regarding the prevalence of Extended spectrum β-lactamases producing *Enterobacteria*. A report claims that on the prevalence of Extended spectrum β-lactamases producing *Enterobacteria* in Adama, Ethiopia was 25% [19], which is comparable with the findings of this study. However, in studies from other parts of Ethiopia like Jimma (38.4%) [20], Addis Ababa (52%) [21] and Harar (33.3%) [22] had reported a higher prevalence of Extended spectrum β-lactamases producing *Enterobacteria* than this study. The lower prevalence of Extended spectrum β-lactamases producing *Enterobacteria* of this study compared with the aforementioned reports might be related to the variability of the sampling

population and methodological difference. Additionally, this study reports the prevalence among HIV patients with both symptomatic and asymptomatic UTI cases that could underestimate the result. In this regard, even if the prevalence found in our study is lower than the studies compared with the general prevalence, HIV patients need special attention as they are immunocompromised and more prone to advance the diseases.

In this study, the very high resistance of microorganisms was reported to amox-clavulanic acid (100%), followed by ampicillin (95.2%), cotrimoxazole (73.8%), cefotaxime (61.9%), and ceftazidime (35.7%) among all isolates in agreement with other reports [20]. This high resistance rate to these drugs might be related to a high rate of prescription of these drugs as a routine therapy and as a prophylaxis therapy in case of cotrimoxazole in HIV/AIDS patients in clinical setups. Additionally, the frequent exposure of HIV/AIDS patients to these antibiotics as a result of frequent infection is related to reduced immunity as well as the increased rate of hospitalization. On the other hand, all isolates were susceptible to nitrofurantoin. This is presumably due to Nitrofurantoin is not a commonly used antibiotic for the empirical treatment of the bacterial infection which preserves the potency of the drug. Hence, it is good if nitrofurantoin is used as an alternative treatment choice in treating resistant isolates.

Regarding multidrug resistance, the overall MDR rate of all isolated *Enterobacteria* in this study was 92.9%. The highest prevalence of MDR might be related to different factors: primarily, due to poor sanitation, inadequate health care services, and poor access to drugs; another factor is related to healthcare provider—they can sell the antimicrobials to patients or receive a kickback fee and other incentives by referring the patients to a particular pharmacy or they might prescribe antibiotics presumptively, even when there is no clinical indication. Additionally, patients believe and perceive that most infections respond to antibiotics and expect to be given antibiotics by healthcare providers even for those nonspecific symptoms that may not be caused by a bacterial infection. Moreover, the cause may be also a high basal level of Multi drug-resistance and antimicrobial-resistance to the general population. As these factors are highly pronounced in HIV/AIDS patients, the rate of MDR might be more pronounced in HIV/AIDS patients [23].

The prevalence of MDR (92.9%) among *Enterobacteria* uropathogens was also assessed in this study. The finding of this study is comparable with the reports in Bahirdar (92.2%) [24] and Hawasa (90.8%) [25]. However, it is higher than the figures reported in Gondar (87.4%) [26] and Dessie (74.9%) [27]. It is also indicated that the prevalence of MDR in this study is higher than other reports like Mozambique (88.2%) in Africa [28] and other countries, such as the United States (19.1%) [29] and Nepal (41.1%) [30]. The variation in the prevalence of MDR *Enterobacteria* isolates could be due to the increasing trend of MDR strains with time and the difference in the study period and study population. Moreover, a higher prevalence of MDR was observed among Extended spectrum β-lactamases producing *Enterobacteria* (100%) than among nonproducing isolates (90.9%) as demonstrated in various reports [20], which might be associated with large plasmids that frequently carry the ESBL marker and are capable of incorporating additional determinants coding for resistance to non-β-lactam antimicrobials. This study has not sought the molecular patterns of Extended spectrum β-lactamases producing *Enterobacteria* isolates to demonstrate which molecular type was prevalent.

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Authors' Contributions
DE: conception of the research idea, study design, data collection, analysis and interpretation, and manuscript write-up. AA: data analysis and interpretation and supervision. FM and SB: data collection and analysis. MB: data analysis and manuscript write-up. SE: conception of research idea, data analysis, and interpretation, supervision, and manuscript write-up. All authors read and approved the final manuscript.

Conflict of Interest
None.

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