Fecal carriage of extended spectrum beta-lactamase producing Enterobacteriaceae among HIV infected children at the University of Gondar Comprehensive Specialized Hospital Gondar, Ethiopia

Biruk Bayleyegn1*, Roman Fisaha2 and Desie Kasew2

Abstract
Background: Human immunodeficiency virus (HIV) and extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae infections are the major challenges in sub-Saharan Africa. Data on the carriage rate of ESBL producing Enterobacteriaceae among HIV infected children is lacking in Ethiopia. Hence this study was aimed to investigate fecal carriage of ESBL producing Enterobacteriaceae among HIV infected children at the University of Gondar comprehensive Specialized Hospital.

Methods: A cross-sectional study was conducted among HIV infected children from January to April 2020. Stool specimens were collected from 161 study participants by convenient sampling and cultured on MacConkey agar. Biochemical identification, antimicrobial susceptibility testing including ESBL production were carried out. Data were analyzed by SPSS version-20 and P-value < 0.05 on multivariate logistic regression analysis was regarded as statistically significant.

Results: From a total of 161 study participants male to female ratio was 1:1.1. Moreover; 96.3% of participants were in HIV stage-I and 90.1% had at least a year highly active antiretroviral therapy exposure. A total of 186 Enterobacteriaceae, with E. coli 60% and K. pneumonia 16.13% predominance were isolated from 161 participants. Majority of isolates were most resistant to amoxicillin (95.1%) and sensitive to CHL (94.1%), CXT (91.4%) and CAZ (91.4%). There were 71(38.17%) multidrug resistant isolates, 13 of which were also ESBL producers. The overall ESBL carriage rate was 32/161 (19.9%). History of antibiotic use was the independent factor associated with ESBL carriage (AOR 3.23 (95% CI 1.054–9.88)) and P-value of 0.04.

Conclusion: ESBL carriage rate of HIV infected children was considerable. Previous antibiotic use was the independent factor. Regular screening for antibiotic resistance on HIV patients before prescription and large-scale antibiotic resistance survey including healthy community may be important.

Keywords: Children, ESBL, Enterobacteriaceae, HIV

Introduction
Human immunodeficiency virus/Acquired immune deficiency syndrome (HIV/AIDS) infection which damages mucous membrane of intestine, baring the
host to antimicrobial resistant infections [1]. Antimicrobial resistance including ESBL production is of significant impact globally mainly in the developing countries [2]. Moreover, the burden of ESBL among healthy people is increasing worldwide with an estimated of 5% rise per year [3]. More than two folds increased prevalence of ESBL producing Enterobacteriaceae in French children between 2010 and 2015 has grown from 4.8 to 10.2% [4]. In addition, a review by Lewis et al. in sub Saharan Africa reported a pooled estimate of ESBL carriage as 18%, indicating the highest burden in the region than reports from US and Europe (3.4 to 7.3%) [5].

ESBL enzymes confer resistance to penicillin, cephalosporin and monobactam [6]. Even some reports indicate the presence of carbapenem resistance [7]. Bacteria produce β-lactamases, a potent family of enzymes that breakdown β-lactam ring of antibiotics making it ineffective and become a major risk in an increasing burden of resistant infections [6]. Multidrug resistance (MDR) was defined as resistance to a minimum of one drug in three classes of antimicrobial agents [8].

East and South Africa region is the largest HIV burden area of the world. Ethiopia, as part of this region contributed to an estimated of 722,248 HIV carriers 2017 [9] and 62,000 children living with HIV/AIDS were reported in 2016 [10]. The immune status of HIV patients is weakened and are at an increased risk of infections, hospitalization and antibiotic consumption than HIV free individuals [11]. Health care associated infection, mechanical ventilation, use of invasive medical devices, prolonged hospital stay and antibiotic use are among factors contributing to increased burden of ESBL producing Enterobacteriaceae [6, 7].

Intestinal carriage of ESBL producing Enterobacteriaceae can cause infections with increased hospital stay, loss of treatment options and associated healthcare costs even leads to death [12]. Hence, the problem needs an emphasis, investigation of ESBL carriage is considered as one of the targets that help to fight antimicrobial resistant infections particularly in resource constrained settings [13]. Data on the carriage rate of ESBL producing Enterobacteriaceae in the general population is very limited in Ethiopia. In spite of their high risk to multiple infections and resulting exposure several antibiotics, the problem among children living with the HIV is overlooked in the country. Hence, this study was aimed to investigate the intestinal carriage rate of ESBL among HIV infected children at the University of Gondar comprehensive Specialized Hospital.

Methods

Study area and population

Institutional cross-sectional study was conducted at the University of Gondar comprehensive Specialized Referral Hospital (UoGCSH) antiretroviral therapy (ART) clinic from January to April 2020. The hospital is found in Gondar town, Amhara region Ethiopia. The town is found at 740 km in the northwest of the capital city, Addis Ababa. The University of Gondar Specialized Referral Hospital is the teaching hospital which provides teaching activities to medical and health science students and the oldest academic institution in Ethiopia. It provides medical, surgical, psychiatric, and many other services to more than 7 million people of the Gondar province and the neighboring regions. The hospital has both inpatient with more than 512 beds and outpatient services. It also provides HIV/AIDS intervention activities like free diagnosis, treatment and monitoring in its ART clinic.

Population

All HIV-infected children who were attending at UoG-SRH ART clinic were the source population while, all HIV infected children who visit UOGRSH ART clinic during the study period were our study population. All HIV infected children who are under 15 years old were included in the study while children without legal guardian or unaccompanied children were excluded from the study.

Socio-demographic and clinical data collection

A pretested questionnaire was employed to collect socio-demographic data of the study participants including age, gender, residence, educational status, family size, family income, family occupation and food habit by face to face interview. In addition, clinical data of the study participants such as, history of invasive medications, World Health Organization (WHO) disease stage of HIV, opportunistic infections, presence of fever and diarrhea, HAART experience and type of HAART, duration of HAART and recent history of antibiotic use were collected by reviewing the medical record of HIV infected children. Anthropometric measurements including weight and height were measured by digital scale. The data collection was investigator administered on site to all participants and/or their guardians at the ART follow up clinic of UoGCSH.

Laboratory procedures

Fresh stool specimen was collected using a coddled clean leak proof plastic cup and transported to Medical microbiology laboratory for culture within 2 h of collection.
Table 1  The Sociodemographic and clinical characteristics of HIV infected children at the University of Gondar Comprehensive Specialized Hospital, 2020

| Variable                                      | Category                        | Frequency (N) | Percent (%) |
|-----------------------------------------------|---------------------------------|---------------|-------------|
| Gender of children                            | Male                            | 77            | 47.8        |
|                                               | Female                          | 84            | 52.2        |
| Age (years) of children                       | Less than or equal to 10        | 37            | 23.0        |
|                                               | 11 and above                    | 124           | 77.0        |
| Residence                                     | Urban                           | 140           | 87.0        |
|                                               | Rural                           | 21            | 13.0        |
| Educational status of children                | No formal education             | 12            | 7.5         |
|                                               | Primary school                  | 130           | 80.7        |
|                                               | Secondary school                | 19            | 11.8        |
| Family income per month                       | Less than 1000                  | 77            | 47.8        |
|                                               | 1000–2000                       | 54            | 33.5        |
|                                               | 2000 birr and above             | 30            | 18.6        |
| Family size                                   | Less than or equal to 4         | 113           | 70.2        |
|                                               | 5 and above                     | 48            | 29.2        |
| Family occupation                             | Privately employed              | 15            | 9.3         |
|                                               | Government worker               | 43            | 26.7        |
|                                               | Merchant                        | 49            | 30.4        |
|                                               | House wife                      | 43            | 26.7        |
|                                               | Other*                          | 11            | 6.8         |
| Family educational status                     | No formal education             | 55            | 34.2        |
|                                               | Primary school                  | 49            | 30.4        |
|                                               | Secondary and above             | 57            | 35.4        |
| HIV status of caregiver                       | Positive                        | 134           | 83.2        |
|                                               | Negative                        | 17            | 10.6        |
|                                               | Not known                       | 10            | 6.2         |
| WHO stage of HIV                              | I                               | 155           | 96.3        |
|                                               | II and late stage               | 6             | 3.7         |
| WAZ                                           | Under weight                    | 11            | 6.8         |
|                                               | Normal                          | 150           | 93.2        |
| HAZ                                           | Stunted                         | 75            | 46.6        |
|                                               | Normal                          | 86            | 53.4        |
| BAZ                                           | Wasted                          | 30            | 18.6        |
|                                               | Normal                          | 131           | 81.4        |
| Opportunistic infections                      | Yes                             | 23            | 14.3        |
|                                               | No                              | 138           | 85.7        |
| Presence of fever                             | Yes                             | 20            | 12.4        |
|                                               | No                              | 141           | 87.6        |
| History of antibiotic use                     | Yes                             | 15            | 9.3         |
|                                               | No                              | 146           | 90.7        |
| Presence of diarrhea                          | Yes                             | 13            | 8.1         |
|                                               | No                              | 148           | 91.9        |
| Viral load                                     | Not detected                    | 61            | 37.9        |
|                                               | Less than or equal to 1000 copies /ml | 44        | 27.3        |
|                                               | Greater than 1000 copies /ml    | 56            | 34.8        |
| HAART experience                              | Less than 6 months              | 9             | 5.6         |
|                                               | 6–12 months                     | 7             | 4.3         |
|                                               | 12 months and above             | 145           | 90.1        |
The collected stool specimen was inoculated on MacConkey agar medium and then incubated at 37°C for 24 h for selective growth of Gram-negative bacteria and lactose fermentation characteristics. Biochemical tests were performed for species identification. Once the species of Enterobacteriaceae were identified, antimicrobial susceptibility test (AST) was performed using Kirby-Bauer Disk Diffusion susceptibility test method on Mueller Hinton agar (MHA). Antimicrobial agents such as ampicillin, amoxicillin-clavulanate, Trimethoprim-Sulfamethoxazole, chloramphenicol, cefixime, cefoxitin, ceftazidime, cefotaxime, tetracycline and ciprofloxacin were used for AST. Discs were selected based on their availability in the local treatment following CLSI-2019. Isolates were also screened for ESBL enzyme production by applying Cefotaxime 30 µg and Ceftazidime 30 µg discs. Phenotypic confirmation of ESBL production was performed using combination disc diffusion method. After screening, simultaneous application of Cefotaxime 30 µg and Ceftazidime 30 µg with their respective combination with clavulanate 10 µg (Cefotaxime–clavulanate 30–10 µg, Ceftazidime–clavulanate 30–10 µg) was used for confirmation following the guideline of Clinical Laboratory Standards Institute (CLSI-2019). Susceptibility was done by preparing suspension of pure isolates comparable to 0.5McFurland standard. The difference in their zone of inhibition were measured after inoculation on MHA and incubation aerobically at 37°C for 16-18hrs. A change in diameter zone of 5 mm and above for either cefotaxime or ceftazidime or both from their combined form was reported as ESBL producing Enterobacteriaceae. Klebsiella pneumoniae ATCC 700,603 (positive control) and Escherichia coli strain ATCC 25,922 (negative control) were used for quality control (CLSI-2019) [14].

### Table 1 (continued)

| Variable                  | Category   | Frequency (N) | Percent (%) |
|---------------------------|------------|---------------|-------------|
| HAART type                | AZT based  | 55            | 34.1        |
|                           | TDF based  | 61            | 37.9        |
|                           | ABC based  | 45            | 28.0        |
| Eating uncooked products  | Yes        | 110           | 68.3        |
|                           | No         | 51            | 31.7        |
| Eating row vegetable      | Yes        | 114           | 70.8        |
|                           | No         | 47            | 29.2        |
| Cytopenia                 | Cytopenia  | 54            | 33.5        |
|                           | Normal     | 102           | 63.4        |

Other*: farmers, daily manual workers, those without work  
WAZ: Weight-for-age; HAZ: Height-for-age; BAZ: Weight-for-Height

**Data analysis**

Data were entered to epi-data version 4.1 and exported to statistical packages for social sciences (SPSS) version-20 for analysis. The results were presented in frequency and percentage through table and text. Univariate and multivariate logistic regression were used to assess the association between the independent variables and the occurrence of ESBL producing Enterobacteriaceae. A variable with P-value of < 0.05 was considered as statistically significant.

**Results**

### Sociodemographic variables of the study population

A total of 161 study participants were recruited in the study with male to female ratio of 1:1.09. Majority of participants were above 10 years (77%), urban residents (87%) and in the WHO HIV stage I (96.3%). Moreover; 46.6% of participants had stunted growth, 34.8% had viral load of > 1000 copies/ml and 90.1% had been taking HAART for at least a year (Table 1).

### Distribution and antimicrobial resistance profile of Enterobacteriaceae

Among 161 study participants who brought stool specimen, there were 186 Enterobacteriaceae isolated from stool culture. *E. coli* was the most common isolate 59.7% followed by *K. pneumoniae* 16.13%. All of isolates were highly resistant to Amoxicillin (95.1%) and Ampicillin (85%). Specifically, more than 96% of *E. coli* and *K. pneumoniae* were resistant to Amoxicillin while, 85.6% *E. coli* and 90% *K. pneumoniae* were resistant to Ampicillin. Similarly, 47.7% of *E. coli* and 46.7% of *K. pneumoniae* were resistant tetracycline. On the other hand, 90%, 92.8%, 97.3% of *E. coli* were sensitive to CAZ, CHL and CXT respectively. High proportion of *K. pneumoniae*
### Table 2. Antimicrobial Susceptibility pattern of Enterobacteriaceae isolates among HIV infected children at the University of Gondar Comprehensive Specialized Hospital, 2020

| Name of Antibiotics | Name of Antibiotics (N) | E. coli (N = 111) | K. pneumoniae (N = 30) | K. zae (N = 4) | Proteus species (N = 4) | Providencia species (N = 3) | Citrobacter species (N = 9) | Enterobacter species (N = 12) | Salmonella species (N = 7) | Shigella species (N = 6) | Row total N (%) |
|---------------------|-------------------------|-------------------|------------------------|---------------|------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------------------------|----------------------|
| AMP                 | S                       | 3 (2.7)           | 1 (3.3)                | 2 (50)        | 2 (16.7)               |                              |                              |                              |                              |                          | 8 (4.3)              |
|                     | I                       | 13 (11.7)         | 2 (6.7)                | 2 (22.2)      | 2 (50)                 | 10 (83.3)                   | 7 (100)                    | 3 (50)                      | 158 (85)                   |                          |                      |
|                     | R                       | 95 (85.6)         | 27 (90)                | 4 (100)       | 3 (100)                | 7 (77.8)                    | 10 (83.3)                   | 7 (100)                    | 3 (50)                      |                          | 20 (10.8)            |
| AMX                 | S                       | 1 (0.9)           | 1 (3.3)                | 2 (50)        | 1 (11.1)               | 1 (8.3)                     |                              |                              | 6 (3.2)                    |                          | 3 (1.6)              |
|                     | I                       | 3 (2.7)           |                       |              |                       |                              |                              | 1 (11.1)                    | 6 (3.2)                    |                          |                      |
|                     | R                       | 107 (96.4)        | 29 (96.7)              | 4 (100)       | 3 (100)                | 8 (88.9)                    | 11 (91.7)                   | 7 (100)                    | 6 (100)                    | 177 (95.1)              |                      |
| AMC                 | S                       | 41 (36.9)         | 11 (36.7)              | 2 (50)        | 1 (25)                 | 2 (66.7)                    | 2 (22.2)                    | 5 (41.7)                    | 3 (50)                      | 68 (36.6)                |                      |
|                     | I                       | 17 (15.3)         | 8 (26.7)               | 1 (25)        | 2 (22.2)               | 1 (14.3)                    | 1 (16.7)                    | 30 (16.1)                   |                            |                          |                      |
|                     | R                       | 53 (47.7)         | 11 (36.7)              | 3 (75)        | 1 (33.3)               | 5 (55.6)                    | 7 (58.3)                    | 4 (57.1)                    | 2 (33.3)                    | 87 (46.8)                |                      |
| CTX                 | S                       | 97 (87.4)         | 26 (86.7)              | 4 (100)       | 2 (66.7)               | 8 (88.9)                    | 8 (66.7)                    | 6 (100)                    | 161 (86.6)                 |                          |                      |
|                     | I                       | 3 (2.7)           | 1 (3.3)                | 1 (8.3)       |                       |                              |                              | 5 (2.7)                    |                            |                          |                      |
|                     | R                       | 11 (9.9)          | 4 (13.3)               | 1 (11.1)      | 3 (25)                 | 1 (14.3)                    | 20 (10.8)                   |                            |                            |                          |                      |
| CAZ                 | S                       | 100 (90)          | 28 (93.3)              | 4 (100)       | 4 (100)                | 3 (100)                     | 8 (88.9)                    | 12 (100)                    | 6 (100)                    | 165 (88.7)              | 91 (9.9)             |
|                     | I                       | 2 (1.8)           | 2 (6.7)                | 1 (11.1)      | 1 (14.3)               | 13 (7)                      |                            |                            |                            |                          |                      |
|                     | R                       | 9 (8.1)           | 2 (6.7)                | 1 (11.1)      | 1 (14.3)               | 2 (1.1)                     |                            |                            |                            |                          |                      |
| CXM                 | S                       | 99 (89.2)         | 27 (90)                | 1 (40)        | 3 (100)                | 6 (66.7)                    | 9 (75)                      | 7 (100)                    | 165 (88.7)                 |                          |                      |
|                     | I                       | 4 (3.6)           | 1 (3.3)                | 1 (8.3)       | 1 (11.1)               | 5 (2.7)                     |                            |                            |                            |                          |                      |
|                     | R                       | 8 (7.2)           | 3 (10)                 | 3 (33.3)      | 3 (25)                 | 17 (9.1)                    |                            |                            |                            |                          |                      |
| CXT                 | S                       | 108 (97.3)        | 25 (83.3)              | 3 (75)        | 3 (100)                | 6 (66.7)                    | 10 (83.3)                   | 7 (100)                    | 171 (91.9)                 |                          |                      |
|                     | I                       | 1 (0.9)           | 2 (6.7)                | 1 (8.3)       |                       | 1 (11.1)                    | 5 (2.7)                     |                            |                            |                          |                      |
|                     | R                       | 2 (1.8)           | 3 (10)                 | 3 (33.3)      | 1 (8.3)                | 10 (5.4)                    |                            |                            |                            |                          |                      |
| CIP                 | S                       | 82 (73.9)         | 22 (73.3)              | 2 (50)        | 7 (77.8)               | 11 (91.7)                   | 5 (71.4)                    | 4 (66.7)                    | 133 (71.5)                 |                          |                      |
|                     | I                       | 22 (19.8)         | 7 (23.3)               | 4 (100)       | 1 (25)                 | 2 (22.2)                    | 2 (28.6)                    | 2 (33.3)                    | 43 (23.1)                  |                          |                      |
|                     | R                       | 7 (6.3)           | 1 (3.3)                | 1 (25)        | 1 (8.3)                | 10 (5.4)                    |                            |                            |                            |                          |                      |
| SXT                 | S                       | 63 (56.8)         | 17 (56.7)              | 2 (50)        | 1 (25)                 | 4 (444)                     | 7 (58.3)                    | 4 (14.3)                    | 46 (66.7)                   | 100 (53.8)               |                      |
|                     | I                       | 3 (2.7)           | 1 (25)                 | 1 (8.3)       | 1 (11.1)               | 5 (2.7)                     |                            |                            |                            |                          |                      |
|                     | R                       | 45 (40.5)         | 13 (43.3)              | 3 (75)        | 2 (66.7)               | 5 (55.6)                    | 3 (33.3)                    | 6 (85.7)                    | 2 (33.3)                    | 81 (43.5)                |                      |
| CHL                 | S                       | 103 (92.8)        | 28 (93.3)              | 4 (100)       | 3 (100)                | 8 (88.9)                    | 12 (100)                    | 7 (100)                    | 175 (94.1)                 |                          |                      |
|                     | I                       | 8 (7.2)           | 2 (1.8)                | 1 (3.3)       | 1 (11.1)               | 11 (5.9)                    |                            |                            |                            |                          |                      |
|                     | R                       | 53 (47.7)         | 14 (46.7)              | 1 (25)        | 3 (75)                 | 3 (33.3)                    | 5 (41.7)                    | 5 (71.4)                    | 3 (50)                      | 87 (46.8)                |                      |

AMP: ampicillin; AMX: amoxicillin; AMC: amoxicillin-clavulanic acid; CTX: cefotaxime; CAZ: ceftazidime; CXM: cefixime; CXT: cefoxitine; CIP: ciprofloxacin; SXT: Trimethoprim-Sulfamethoxazole; CHL: chloramphenicol; TET: tetracycline
were resistant to CXM (90%), CAZ (93.3%) and CHL (93.3%) (Table 2).

Distribution of MDR and ESBL producing Enterobacteriaceae

The proportion of MDR from the total Enterobacteriaceae isolates was 71/186 (38.7%) in this study. Of the MDR isolates, 46/71 (64.8%) were *E. coli* followed by 8/71 (11.3%) *K. pneumonia*. Moreover, thirteen of the MDR isolates (twelve *E. coli* and one *K. pneumonia*) were ESBL producers. Of the total (161) participants 19.9% were carriers of ESBL producing isolates. There was no double or multiple carriage of ESBL isolates. All ESBL producing isolates were *E. coli* (16.2%) and *K. pneumonia* (3.7%) (Table 3).

Factors associated with ESBL production

Among the factors analyzed in bivariate logistic regression, family education and history of antibiotic use had P-value of less than 0.2 and were fitted to multivariate analysis. But in multivariate analysis, only history of antibiotic use had statistically significant association with ESBL carriage (AOR, 3.23 95% CI 1.054–9.88) and P-value of 0.04 (Table 4).

Discussion

The overall carriage rate of ESBL producing Enterobacteriaceae among children living with HIV was 19.9% (95% CI 14.4–26.1). The result was comparable to reports among healthy children (16%) [15] and (22%) [16] in Cameroon, 21% Madagascar [17], (24.8%) Lebanon [18] and 16.8% Sweden [19]. On the other hand, the result was lower compared to reported results 31% in Niger [20], 34.3% Tanzania [21], 59% Central Africa Republic [22], 32.6% Guinea Bissau [23] and 49.6% Lebanon [24]. The fecal carriage of ESBL producing Enterobacteriaceae in this study was a little higher than 13.7% in Zimbabwe [25] and higher than reports among HIV negative children from different geographic regions: (10%) in Kenya [26], (5.0%) Ghana [27], (3.5%) United States of America [28], (4.6%) France [29], (4.7%) South Africa [12]. Geographic variation, variation in the method of ESBL detection or measures taken for the prevention of antimicrobial resistance might be responsible for the difference.

The rate of antimicrobial resistance in this study were highest against Amoxicillin (95.1%), Ampicillin (85%), Amoxicillin-clavulanic acid (46.8%), Tetracycline (46.8%) and Trimethoprim-sulfamethoxazole (43.5%). However; lowest resistance to ceftazidime (91.9), cefoxitin (91.9%) and chloramphenicol (94.1%) was observed. The result concords to a report from Arba Minch, Ethiopia [30]. This high proportion of resistance indicates isolates could probably adapt themselves to the commonly prescribed antibiotics. Trimethoprim-sulphamethoxazole is a prophylactically prescribed to HIV infected children in Ethiopia as per WHO recommendations to resource limited settings [31]. Comparable resistance to trimethoprim-sulphamethoxazole was reported in Nepal 48.9% [32], Nigeria 54% [33]. But resistance rate found in this study was lower than 91.3% in Madagascar [17]. The difference might be due to difference in population.

The overall MDR rate of Enterobacteriaceae isolates among HIV infected children were 71/186 (38.2%). The two most common MDR isolates were *E. coli* 46/71 (64.8%) and *K. pneumonia* 8/71 (11.3%). In addition, 13/71 (18.3%) MDR isolates (twelve *E. coli* and one *K. pneumonia*) were ESBL producers. This result was lower than 68.3% MDR in Addis Ababa, Ethiopia [34].

### Table 3 Frequency of Enterobacteriaceae and distribution of MDR and ESBL producing isolates among HIV infected children at the University of Gondar Comprehensive Specialized Hospital, 2020

| Species of isolate | Total No (%) of isolates | No of MDR isolates | % of MDR isolates, N (71) | % MDR from total isolates (186) | % ESBL from total sample size (32/161) | Both MDR and ESBL 13/186 |
|--------------------|-------------------------|--------------------|---------------------------|---------------------------------|---------------------------------------|--------------------------|
| E. coli            | 111 (59.7)              | 46                 | 64.8                      | 24.7                            | 26 (16.2)                             | 12 (6.5)                 |
| K. pneumonia       | 30 (16.1)               | 8                  | 11.3                      | 4.3                             | 6 (3.7)                               | 1 (0.5)                  |
| Kazenia            | 4 (2.2)                 | 1                  | 1.4                       | 0.5                             | –                                     | –                        |
| Proteus species    | 4 (2.2)                 | 3                  | 4.2                       | 1.6                             | –                                     | –                        |
| Citrobacter species| 9 (4.8)                 | 4                  | 5.6                       | 2.2                             | –                                     | –                        |
| Enterobacter species| 12 (6.5)              | 2                  | 2.8                       | 1.1                             | –                                     | –                        |
| Salmonella species | 7 (3.8)                 | 4                  | 5.6                       | 2.2                             | –                                     | –                        |
| Shigella species   | 6 (3.2)                 | 2                  | 2.8                       | 1.1                             | –                                     | –                        |
| Providencia species| 3 (1.6)                 | –                  | –                         | –                               | –                                     | –                        |
| Total              | 186 (100)               | 71                 | 100                       | 38.7                            | 19.9%                                 | 7%                       |
Table 4  Factors associated with ESBL Production among HIV infected children at the University of Gondar Comprehensive Specialized Hospital, 2020

| Variable                        | Category                      | ESBL carriage | COR (95% CI) | P-value | AOR (95% CI) | P-value |
|--------------------------------|-------------------------------|---------------|--------------|---------|--------------|---------|
|                                |                               | Yes N (%)     | N (%)        |         |              |         |
| Gender                         | Male                          | 13 (16.9)     | 64 (83.1)    | 1       |              |         |
|                                | Female                        | 18 (21.4)     | 66 (78.6)    | 1.34 (0.61–2.96) | 0.47 |
| Age (years)                    | Less than or equal to 10      | 7 (18.9)      | 30 (81.1)    | 1       |              |         |
|                                | 11–15                         | 24 (19.4)     | 100 (80.6)   | 1.03 (0.40–2.62) | 0.95 |
| Residence                      | Urban                         | 28 (20)       | 112 (80)     | 1       |              |         |
|                                | Rural                         | 3 (14.3)      | 18 (85.7)    | 0.67 (0.18–2.42) | 0.54 |
| Family income per month        | Up to 1000 ETB                | 15 (19.5)     | 62 (80.5)    | 0.97 (0.34–2.79) | 0.98 |
|                                | 1000–2000 ETB                 | 10 (18.5)     | 44 (81.5)    | 0.91 (0.29–2.81) | 0.87 |
|                                | Above 2000 ETB                | 6 (20)        | 24 (80)      | 1       |              |         |
| Family size                    | Down to 4                     | 21 (18.6)     | 92 (81.4)    | 1       |              |         |
|                                | S–8                           | 10 (20.8)     | 38 (79.2)    | 1.15 (0.50–2.68) | 0.74 |
| Family educational status      | No                            | 6 (10.9)      | 49 (89.1)    | 0.46 (0.16–1.33) | 0.15 |
|                                | Primary school                | 13 (26.5)     | 36 (73.5)    | 1.35 (0.55–3.33) | 0.51 |
|                                | Secondary school & above      | 12 (21.1)     | 45 (78.9)    | 1       |              |         |
| WHO stage                      | I                             | 29 (18.7)     | 126 (81.3)   | 1       |              |         |
|                                | II                            | 2 (33.3)      | 4 (66.7)     | 2.17 (0.38–12.44) | 0.38 |
| BAZ                            | Underweight                   | 8 (26.7)      | 22 (73.3)    | 1.71 (0.68–4.31) | 0.26 |
|                                | Normal                        | 23 (17.6)     | 108 (82.4)   | 1       |              |         |
| HAZ                            | Stunted                       | 12 (16)       | 63 (84)      | 1.45 (0.67–3.32) | 0.33 |
|                                | Normal                        | 19 (22.1)     | 67 (77.9)    | 1       |              |         |
| Presence of fever              | Yes                           | 4 (20)        | 16 (80)      | 1.06 (0.33–3.41) | 0.93 |
|                                | No                            | 27 (19.1)     | 114 (80.9)   | 1       |              |         |
| Opportunistic infections       | Yes                           | 6 (26.1)      | 17 (73.9)    | 1.60 (0.57–4.45) | 0.37 |
|                                | No                            | 25 (18.1)     | 113 (81.9)   | 1       |              |         |
| Diarrhea                       | Yes                           | 3 (23.1)      | 10 (76.9)    | 1.29 (0.33–4.98) | 0.72 |
|                                | No                            | 28 (18.9)     | 120 (81.1)   | 1       |              |         |
| History of antibiotic use      | Yes                           | 6 (40)        | 9 (60)       | 3.23 (1.05–9.88) | 0.04 |
|                                | No                            | 25 (17.1)     | 121 (82.9)   | 1       |              | 0.04 |
| Eating animal products         | Yes                           | 24 (21.8)     | 86 (78.2)    | 1.75 (0.70–4.39) | 0.23 |
|                                | No                            | 22 (18)       | 86 (82)     | 1       |              |         |
| Eating green vegetables        | Yes                           | 23 (20.2)     | 91 (79.8)    | 1.23 (0.51–2.99) | 0.65 |
|                                | No                            | 8 (17)        | 39 (83)      | 1       |              |         |
| Cytopenia                      | Normal                        | 21 (19.6)     | 86 (80.4)    | 1       |              |         |
|                                | Cytopenia                     | 10 (18.5)     | 44 (81.5)    | 0.93 (0.40–2.14) | 0.87 |
| Viral load                     | ≤ 1000 copy /ml               | 6 (13.6)      | 16 (86.4)    | 0.53 (0.19–1.51) | 0.24 |
|                                | > 1000 copy /ml               | 11 (19.6)     | 45 (80.4)    | 0.82 (0.34–2.00) | 0.66 |
|                                | Not detected                  | 14 (23)       | 47 (77)      | 1       |              |         |
| HAART type                     | AZT based                     | 10 (18.2)     | 45 (81.8)    | 0.78 (0.29–2.08) | 0.62 |
|                                | TDF based                     | 11 (18)       | 50 (82)      | 0.77 (0.29–2.01) | 0.59 |
|                                | ABC based                     | 10 (22.2)     | 35 (77.8)    | 1       |              |         |

Bold numeral represented statistically significant
This may be due to the difference in the study population and this ESBL producing Enterobacteriaceae may transfer their resistant trait to the naïve enteric commensals.

Among the total 161 HIV infected children included in this study, 32 (19.9%) were carriers of phenotypically confirmed ESBL producing Enterobacteriaceae. The result was higher than 5.3% prevalence in Uganda [35] but lower than 28.46% in Nepal [32]. In this study there was no double or multiple carriage of ESBL isolates and all of the ESBL producing isolates were E. coli 26 (16.2%) and K. pneumonia 6 (3.7%). This high resistance to multiple antimicrobial agents in addition to ESBL production is a bottleneck in the treatment of infectious diseases and pushes to the utilization of last resort drugs resulting in loss of effective treatment option [36]. Over use, frequent and intermittent use together with ease of access to the antibiotics without prescription from private pharmacies could be the possible rationale for the increased resistance.

The history of antibiotic use has shown statistically significant association. The ESBL carriage rate among children with drug use was more than 3 times compared to their counter parts (AOR 3.2, 95% CI 1.05–9.9). History of antibiotic use is also reported as a risk factor by several studies too [15, 21]. A study also has reported high family income as an independent factor associated with increased risk of ESBL carriage. Because low family income limits the rate of exposure to antibiotics which intern reduces the risk of antibiotic resistance and ESBL carriage [22]. On the other hand, low family income was reported as significant factor associated with increased risk of ESBL carriage [21]. The result of this study in contrast showed that ESBL producing isolates were distributed irrespective of the income level and no significant association was found. Family size appeared to be associated with ESBL carriage in bivariate analysis but in multivariate analysis it was not significantly associated. Residence was reported as a factor by a study done among Hospitalized patients at Arba Minch, Ethiopia [30]. But it didn’t show statistically significant association with ESBL carriage in this study. In addition, in this study, age was not significantly associated with ESBL carriage which is in agreement with a report in Madagascar [17]. This study included only HIV infected children who had visited the hospital during the study period. Advanced techniques of ESBL detection were not used in this study.

**Conclusion**

Fecal carriage of MDR and ESBL producing Enterobacteriaceae among HIV infected children was considerable. The History of antibiotic use was the independent factor associated with the carriage of ESBL producing Enterobacteriaceae. Regular screening of HIV patients for the carriage of ESBL producing isolates need to be strengthened. In addition, large scale antibiotic resistance survey including healthy community could be important.

**Abbreviations**

AOR: Adjusted odds ratio; ART: Antiretroviral therapy; AST: Antimicrobial susceptibility test; ATCC: American type culture collection; CLSI: Clinical Laboratory Standard Institute; ESBL: Extended spectrum beta lactamase; HAART: Highly active antiretroviral therapy; HIV/AIDS: Human immunodeficiency virus/Acquired immune deficiency syndrome; MDR: Multidrug resistance; MHA: Mueller Hinton Agar; UoGCSH: University of Gondar comprehensive Specialized Referral Hospital; WHO: World Health Organization.

**Acknowledgements**

We would like to acknowledge the study participants who gave us their stool specimen with free consent voluntarily and with trust on us. We also want to extend our gratitude to the staff members working in Medical microbiology laboratory for the material and technical support.

**Authors’ contributions**

DK: Conception of the research idea, laboratory investigation, data analysis, writing of the manuscript; RF: laboratory investigation, resource acquisition, data editing and analysis, supervision; BB: Conception of the research idea, data collection, supervision, review of the manuscript. All authors read and approved the final manuscript.

**Funding**

No special fund obtained.

**Availability of data**

All the data are incorporated in the manuscript.

**Declarations**

**Ethical approval and consent**

The study was conducted after ethical approval was obtained from Ethical review committee of school of Biomedical and Laboratory science, college of medicine and health sciences, university of Gondar. The study was conducted after written consent and/or assent of study participants and/or their guardians was obtained.

**Consent for publication**

All the authors have reviewed the manuscript and agreed to submit.

**Competing interests**

All authors have agreed no any conflict of interest.

**Author details**

1. Department of Clinical Hematology and Immunohematology, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia.
2. Department of Medical Microbiology, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia.

Received: 18 November 2020    Accepted: 12 April 2021
Published online: 21 April 2021
References

1. Nwosu FC, Avershina E, Wilson R, Rudi K. Gut microbiota in HIV infection: implication for disease progression and management. Gastroenterol Res Pract. 2014;2014:483185.

2. Subramanya SH, Baiy J, Nayak N, Padukone S, Sathian B, Gokhale S. Low rate of gut colonization by extended-spectrum β-lactamase producing Enterobacteriaceae in HIV infected persons as compared to healthy individuals. J Antimicrob Chemother. 2015;70(9):2334–40.

3. Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal colonization with extended-spectrum beta-lactamase–producing Enterobacteriaceae and risk factors among healthy individuals: a systematic review and meta-analysis. Rev Infect Dis. 2016,63(3):310–8.

4. Birgy A, Levy C, Bidet P, Thollot F, Derk V, Béchet S, et al. ESBL-producing Escherichia coli ST131 versus non-ST131: evolution and risk factors of carriage among French children in the community between 2010 and 2015. J Antimicrob Chemother. 2016;71(10):3449–56.

5. Lewis JM, Lester R, Garner P, Feasey NA. Gut mucosal colonisation with extended-spectrum beta-lactamase–producing Enterobacteriaceae in sub-Saharan Africa: a systematic review and meta-analysis. Wellcome Open Res. 2019;4:160.

6. Lukac PJ, Bonomo RA, Logan LK. Extended-spectrum β-lactamase–producing Enterobacteriaceae in children: old foe, emerging threat. Clin Infect Dis. 2015;60(9):1389–97.

7. Hu YJ, Ogyu A, Cowling BJ, Fukuda K, Pang HH. Available evidence of antibiotic resistance from extended-spectrum β-lactamase-producing Enterobacteriaceae in paediatric patients in 20 countries: a systematic review and meta-analysis. Bull World Health Organ. 2019;97(7):486.

8. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.

9. Kibret GD, Ferede A, Leshargie CT, Wagnew F, Ketema DB, Alebel A. Trends and spatial distributions of HIV prevalence in Ethiopia. Infect Dis Poverty. 2019;8(1):90.

10. Girum T, Wasie A, Worku A. Trend of HIV/AIDS for the last 26 years and predicting achievement of the 90–90-90 HIV prevention targets by 2020. In: 2020. In: 2020:19(1):915.

11. Hosur Subramanya S, Baiy J, Nayak N, Padukone S, Sathian B, Gokhale S. Low rate of gut colonization by extended-spectrum β-lactamase-producing Enterobacteriaceae in HIV infected persons as compared to healthy individuals in Nepal. PLoS ONE. 2019;14(2):e0212208.

12. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.

13. Control C, Prevention. Antibiotic resistance threats in the United States, 2013: Centre for Disease Control and Prevention, US Department of Health and . . ., 2013. 14. Weinstein MP. Performance standards for antimicrobial susceptibility testing: Clinical and Laboratory Standards Institute, 2019.

15. Lonchel CM, Meex C, Gangué-Piéboji J, Boreux R, Assoumou M-C, Gangoué-Piéboji J, Boreux R, Assoumou M-CO, Gangoué-Piéboji J, Boreux R, Assoumou M-CO. Gut microbiota in HIV-infected children with severe acute malnutrition in Niger. Clin Infect Dis. 2011;53(7):677–85.

16. Tellevik MG, Blomberg B, Kommel Ø, Maselle SY, Langeland N, Moyo SJ. High prevalence of faecal carriage of ESBL-producing Enterobacteriaceae among children in Dar es Salaam, Tanzania. PLoS ONE. 2011;6(12):e0168024.

17. Faria A, Frank T, Tondeur L, Bata P, Goody J, Onambele M, et al. High rate of faecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae in healthy children in Bangui, Central African Republic. Clin Microbiol Infect. 2016;22(10):891.

18. Kanh L, Kumar G, Keswani T, Bhattacharyya A, Chandar SS, Rao KB. Protease inhibitors from marine actinobacteria as a potential source for antimalarial compound. PLoS ONE. 2014;9(3):e95072.

19. Hijazi SM, Fawzi MA, Ali FM, Abd El Galil KH. Multidrug-resistant ESBL-producing Enterobacteriaceae and associated risk factors in community infants in Lebanon. J Infect Develop Ctries. 2016;10(9):947–55.

20. Wilmore SS, Kranzer K, Williams A, Makumare B, Nhizda A, Mayini J, et al. Carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in HIV-infected children in Zimbabwe. J Med Microbiol. 2017;66(5):609.

21. Kajjo N, Kosige P, Ooko M, Wafala L, Muntu N, Anampuya K, et al. Carriage and acquisition of extended-spectrum β-lactamase–producing Enterobacteriales among neonates admitted to hospital in Kilifi. Kenya Clin Infect Dis. 2019. 2019;69(5):751–9.

22. Sanneh B, Kebbeh A, Jallow HS, Camara Y, Mwamakamba LW, Ceesay IF, et al. Prevalence and risk factors for faecal carriage of extended spectrum β-lactamase producing Enterobacteriaceae among food handlers in lower basic schools in West Coast Region of The Gambia. PLoS ONE. 2018;13(8):e0208089.

23. Islam S, Selvarangan R, Kanwar N, McHenry R, Chappell JD, Halasa N, et al. Intestinal carriage of third-generation cephalosporin-resistant and extended-spectrum β-lactamase-producing Enterobacteriaceae in healthy US children. J Pediatr Infect Dis Soc. 2018;7(3):234–40.

24. Birgy A, Cohen R, Levy C, Bidet P, Courroux C, Benani M, et al. Community faecal carriage of extended-spectrum beta-lactamase–producing Enterobacteriaceae in French children. BMC Infect Dis. 2012;12(1):315.

25. Aklilu A, Maniall A, Ameya G, Woldemariam M, Siraj M. Gastrointestinal tract colonization rate of extended-spectrum beta-lactamase-and carbapenemase-producing Enterobacteriaceae and associated factors among hospitalized patients in Arba Minch General Hospital, Arba Minch. Ethiop Infect Drug Resist. 2020;13(1):157–26.

26. WHO. Co-trimoxazole prophylaxis for HIV-exposed and HIV-infected infants and children: practical approaches to implementation and scale up. Geneva: World Health Organization, 2009.

27. Mandal DK, Sah SK, Mishra SK, Sharma S, Kattel HP, Pandit S, et al. Multidrug-resistant ESBL- and AmpC-β-lactamase-producing Enterobacteriaceae (ESBL-PE) in Healthy Community and Outpatient Department (OPD) patients in Nepal. Can J Infect Dis Med Microbiol. 2020;2020:5154217.

28. Ogwuatu CC, Ogunsola F, Iwuafor A, Akujobi C, Egwuatu T, Nnachi A, et al. Effect of trimethoprim-sulfamethoxazole prophylaxis on faecal carriage rates of resistant isolates of Escherichia coli in HIV-infected adult patients in Lagos, Afr. J Infect Dis. 2017;11(1):18–25.

29. Teklu DS, Negeri AA, Legese MH, Redada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. Antimicrob Resist Infect Control. 2019;8(1):39.

30. Najjuka CF, Kateete DP, Kajumbula HM, Joloba ML, Essack SY. Antimicrobial susceptibility profiles of Escherichia coli and Klebsiella pneumoniae isolated from outpatients in urban and rural districts of Uganda. BMC Res Notes. 2016;9(1):235.

31. Pitout JD. Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae. Drugs. 2010;70(3):313–33.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.