Extended-spectrum beta-lactamase production and multi-drug resistance among *Enterobacteriaceae* isolated in Addis Ababa, Ethiopia

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**Abstract**

**Background:** The global emergence and spread of extended-spectrum beta-lactamases (ESBLs) producing *Enterobacteriaceae* have been threatening the ability to treat an infection. Hence, this study aimed to determine the prevalence of ESBL-producing and multi-drug resistance (MDR) *Enterobacteriaceae* (ESBLs-E) from different clinical specimens in Addis Ababa, Ethiopia.

**Methods:** A cross-sectional study was conducted from January 1 to May 30, 2017. A total of 426 *Enterobacteriaceae* isolates were identified from clinical specimens. The isolates were collected from four laboratories. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Muller Hinton agar (MHA). All *Enterobacteriaceae* were screened for ESBLs production using cefotaxime and ceftazidime as per CLSI guideline. Each ESBL screening positive *Enterobacteriaceae* were confirmed by a combination disk test (CDT). Data were entered and analyzed by using SPSS version-20.

**Result:** The most frequent *Enterobacteriaceae* were *E. coli* 228 (53.5%) and *K. pneumoniae* 103 (24.1%). The magnitude of ESBLs-E was 57.7% (246/426). The highest frequencies of ESBLs-E were observed in blood specimens (84.4%) and the highest ESBLs production was observed in *K. pneumoniae* (85.4%). The highest resistance level was seen to sulfamethoxazole-trimethoprim (77.0%), amoxicillin with clavulanic acid (71.6%), cefotaxime (62.2%), cefepime (60.3%) and ceftazidime (60.8%). The overall magnitude of multi-drug resistance (MDR) level was 68.3%. Of ESBLs-E, 96.3% of them were MDR (*P* < 0.001).

**Conclusion:** There was a high prevalence of ESBLs-E and MDR isolate in Addis Ababa. Most of ESBLs-E was isolated primarily in blood and urine. The highest ESBLs production was observed among *K. pneumoniae*. Hence, strong infection control strategies must be implemented in hospital settings of the country.

**Keywords:** ESBLs, MDR, *Enterobacteriaceae*, Clinical specimens, Addis Ababa, Ethiopia
Introduction

Enterobacteriaceae are Gram-negative, facultative anaerobes, and non-sporing bacilli. These bacteria have become one of the most important causes of nosocomial and community-acquired infections. They can cause urinary tract, respiratory tract, and bloodstream and wound infections. Increasing rates of antimicrobial resistance have become a worldwide problem predominantly caused by Gram-negative bacteria, the Enterobacteriaceae [1, 2].

Beta-lactam drugs such as extended-spectrum penicillins, cephalosporins, monobactams, carbapenems, fluoroquinolones (e.g. ciprofloxacin) and aminoglycosides (e.g. gentamicin) are among the most prescribed antibiotics to treat infections caused by Enterobacteriaceae. Despite the in vivo efficacy and/or toxicity of these antibiotics (e.g. carbapenems, colistin, tigecycline) are available to treat infection caused by Enterobacteriaceae, although the in vivo efficacy and/or toxicity of these drugs is not well-known [13, 14].

The widespread use of beta-lactam antibiotics has caused the expansion of resistant Enterobacteriaceae. The most important mechanism of resistance to beta-lactam antibiotics involves the production of beta-lactamases (especially extended-spectrum beta-lactamases) that inactivate beta-lactam antibiotics and this continue to be the prominent cause of beta-lactam antibiotics resistance among Enterobacteriaceae worldwide. ESBL-producing Enterobacteriaceae are important members of antibiotic-resistant bacteria that cause hospital and community-acquired infections [3, 4].

ESBL is an enzyme that is produced by bacteria to become resistant to extended-spectrum penicillins, cephalosporins, and monobactams except for cephamycins and carbapenems. It is also inhibited by beta-lactamase inhibitors like clavulanic acid. A worrisome increasing trend has been reported on the development of resistance to extended-spectrum cephalosporins caused by ESBL producing Enterobacteriaceae [2, 3, 5]. Among Enterobacteriaceae, ESBLs have been found mostly in Klebsiella spp. and E. coli as well as in other Enterobacteriaceae families such as Enterobacter spp., Proteus spp., Citrobacter spp., Morganella spp., Providencia spp., Salmonella spp., and Serratia spp [6–8].

Being plasmid mediated, ESBL is easily transmitted among members of Enterobacteriaceae. The dissemination of this resistance applies not only to beta-lactams but also to other commonly used antibiotics such as fluoroquinolones, aminoglycosides, and sulphonamides [9, 10]. Consequently, many patients need the ‘last resort’ antibiotics treatment such as carbapenems [2, 11]. Again the use of carbapenems has led to the rapid selection of carbapenem-resistant Enterobacteriaceae [12]. Only a few antibiotics (e.g. carbapenems, colistin, tigecycline) are available to treat infection caused by ESBL-producing bacteria, although the in vivo efficacy and/or toxicity of these drugs is not well known [13, 14].

Assessing ESBL producing Enterobacteriaceae in the local scenario is necessary to understand the epidemiology and the disease burden as well as to design and implement hospital infection control strategies to prevent the further occurrence and spread of such bacteria. However, little is known about the magnitude of ESBL producing Enterobacteriaceae in Addis Ababa, Ethiopia. Moreover, to the best of our knowledge, almost all clinical bacteriology laboratories in Ethiopia do not perform ESBL tests. Hence, this study aimed to determine the prevalence of ESBL producing and MDR Enterobacteriaceae in different clinical specimens in Addis Ababa, Ethiopia.

Methods

Study setting

A laboratory-based cross-sectional study was conducted from January to May 2017 at the Ethiopian Public Health Institute (EPHI) Clinical Bacteriology and Mycology National Reference Laboratory in Addis Ababa. This laboratory is Ethiopia’s main referral laboratory and is accredited by the Ethiopian National Accreditation Office (ENAO). The Enterobacteriaceae isolates used for this study were collected from four microbiology laboratories: EPHI clinical bacteriology laboratory, International Clinical Laboratories (ICL), Tikur Anbessa Specialized Hospital (TASH), and Yekatit 12 Medical College Hospital Microbiology Laboratory. The isolates were collected using a convenient sampling technique. All consecutive Enterobacteriaceae isolated from clinical specimens in the selected bacteriology laboratories were included in the study. Demographic characteristics of the patients were recorded using a pre-developed worksheet. The isolates were collected using Tryptose Soy Broth (TSB) (Oxoid Ltd., Basingstoke, United Kingdom) containing 20% glycerol and temporarily stored at −20 °C in the respective laboratory. Within a week the isolates were transported to the EPHI clinical bacteriological laboratory using a cold box with ice.

Culture and identification

The isolates preserved at −70 °C were recovered by re-suspension of the stored isolate in Tryptose Soy Broth (Oxoid Ltd., Basingstoke, United Kingdom). After a few hours, the isolates were inoculated and incubated on MacConkey agar (Oxoid Ltd., Basingstoke, United Kingdom) at 37 °C for 18–24 h. After incubation, the colony was characterized by colony appearance, Gram stain, and biochemical tests. The isolates were identified by standard microbiological laboratory methods [15]. Antibiotic susceptibility and ESBLs confirmatory tests were done using the pure isolate sub-cultured on to 5% sheep blood agar (HiMEDIA Laboratories Pvt. Ltd., Mumbai, India).
Preparation of clavulinate stock solution
For the combination disk test CDT method, the combined disks (Ceftazidime-clavulanate (30 µg/10 µg), and cefotaxime-clavulanate (30 µg/10 µg) disks) were prepared from in-house made clavulanate solution according to CLSI guideline [16]. From potassium clavulanate analytical standard powder (Sigma-Aldrich Corp, St. Louis, MO USA) stock solution of clavulanate at 1000 µg/ml was prepared, aliquoted, and stored at – 70 °C. When we were ready to perform CDT (each day of testing), 10 µL of clavulanate solution was added to ceftazidime (30 µg) and cefotaxime (30 µg) disks (Abtek Biologicals Ltd., Liverpool, United Kingdom) and we allowed about 30 min for the clavulanate to absorb, and the disks to be dry enough for application. The combined disks were used immediately (within 30 min) after they had dried.

Antibiotic susceptibility testing
Antimicrobial susceptibility testing was carried out by the Kirby-Bauer disc diffusion method and the results were expressed as susceptible, intermediate or resistant according to CLSI guideline [16]. After preparation of 0.5 McFarland turbidity inoculums, Muller-Hinton Agar (MHA) (Oxoid LTD, Basingstoke, Hampshire, United England) plates were inoculated and antimicrobial discs were applied to the plate. The antibiotic discs used in this study were amoxicillin-clavulanic acid (AMC: 20/10 µg), cefotaxime (CTX: 30 µg), ceftazidime (CAZ: 30 µg), cefepime (FEP: 30 µg), Cefoxitin (30 µg), meropenem (MER: 10 µg), gentamicin (GEN:10 µg), amikacin (30 µg) ciprofloxacin (CIP: 5 µg), norfloxacin (NOR: 10 µg) and sulfamethoxazole-trimethoprim (SXT: 3.75/1.25 µg). The antibiotic discs used were from Abtek Biologicals Ltd., Liverpool, United Kingdom product. An Enterobacteriaceae isolate was considered as MDR if it was non-susceptible to three or more drugs from different classes/groups of antibiotics [17].

Screening for potential ESBL-producing isolate
The isolates that showed an inhibition zone size of ≤22 mm with ceftazidime (30 µg) and/or ≤27 mm with cefotaxime (30 µg) were considered as potential ESBL-producer (screening ESBL positive) and were selected for confirmation for ESBLs production using CDT as recommended by CLSI guideline [16].

Confirmation of ESBLs with combination disc test
A disc of ceftazidime (30 µg), cefotaxime (30 µg) and cefepime (30 µg), and ceftazidime + clavulanic acid (30 µg/10 µg), cefotaxime (30 µg) + clavulanic acid (30 µg/10 µg) and cefepime (30 µg) + clavulanic acid (30 µg/10 µg) was placed at appropriate distance on a MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight (18–24 h) at 37 °C. Cefepime (30 µg) and cefepime (30 µg) + clavulanic acid (30 µg/10 µg) is EUCAST’s recommendation. An increase in the inhibition zone diameter of > 5 mm for a combination disc versus ceftazidime or cefotaxime disc alone was confirmed as ESBLs production [16, 18].

Quality control and data quality assurance
Quality control for the new batch was performed using ATCC 25922 E. coli standard strain to check the quality of culture media and antibiotics disks. For the ESBL confirmatory test, K. pneumoniae ATCC® 700603 (ESBLs positive) and E. coli ATCC® 25922 (ESBLs negative) control strains were used to check the quality of the commercially purchased antibiotics disks and in-house prepared combination disks [16]. The data collection form was checked for its completeness and accuracy before recording the data. Culture and antibiotics susceptibility test results were recorded carefully before entry to SPSS software (version 20).

Data entry and analysis
Data were entered and analyzed using SPSS software (version 20). Proportions and the actual number of ESBL-producing Enterobacteriaceae isolates were used to describe frequency outputs for categorical variables. The data were presented in table and graphs. Mean and standard deviation were used to describe continuous variables.

Results
Demographic characteristics of the patients
A total of 426 consecutive non-repetitive Enterobacteriaceae isolates were collected from the four microbiology laboratories from January 1 to May 30, 2017. During the study period, we obtained 150 isolates from International Clinical Laboratories (ICL), 118 isolates from Tikur Anbessa Specialized Hospital (TASH), 89 isolates from Clinical Bacteriology and Mycology National Reference Laboratory in Ethiopian Public Health Institute (EPHI), and 69 isolates from Yekatit 12 Medical College Hospital (YMCH). These isolates were identified from different clinical specimens: 272 from urine; 90 from blood; 40 from pus; 11 from body fluids; 6 from sputum; 3 from ear discharge; 2 from eye discharge; and 2 from cerebrospinal fluid (CSF) (Table 1).

Among the patients included in the study, 236 (54.4%) of the isolates were recovered from males and 190 (44.6%) from females. The most frequently isolate found among males were E. coli (47.4%) and K. pneumoniae (28.4%), and among females were E. coli (58.5%), K. pneumoniae (20.8%). The isolates were obtained from patients aged from 1 day to 91 years with the mean age of 32.6 years (standard deviation 25.6). Among all Enterobacteriaceae isolates, 58/426 (13.6%) were isolated...
from infants less than 1 year, 93/426 (21.8%) from children less than 5 years, and 135/426 (31.7%) from children less than 15 years of age (Table 1).

**Frequency of Enterobacteriaceae isolates**

Among all Enterobacteriaceae, the most frequent isolates were *E. coli* (53.5%; 228/426) and *K. pneumoniae* (24.1%; 103/426). *E. coli* were predominantly isolated in urine (82.5%; 188/228) and in blood specimens (10.5%; 24/228). From the total *K. pneumoniae* isolate, 54.1% (53/103) were obtained from blood, 31.1% (32/103) from urine and 11.6% (12/103) wound/pus. Furthermore, from all *K. pneumonia* 50.5% (50/103) were isolated from children age less than 15 years (Table 1).

**Antibiotics resistance pattern of Enterobacteriaceae**

The antibiotics resistance pattern of *Enterobacteriaceae* isolated in different clinical specimens against 11 antibiotics is presented in Table 2. The highest resistance level was recorded for sulfamethoxazole-trimethoprim (77.0%), followed by amoxicillin-clavulanic acid (70.0%), meropenem (64.3%), and ciprofloxacin (64.0%). In addition, its resistance level to cefotaxime, cefepime, and ceftazidime was 54.8, 53.5, and 53.1% respectively. However, the lowest level of resistance was observed to meropenem (3.5%) and amikacin (13.8%).

*E. coli* showed the highest resistance to sulfamethoxazole-trimethoprim (77.6%) followed by amoxicillin-clavulanic acid (70.0%), norfloxacin (64.3%), and ciprofloxacin (64.0%). In addition, its resistance level to cefotaxime, cefepime, and ceftazidime was 54.8, 53.5, and 53.1% respectively. However, the lowest level of resistance was observed to MER (3.5%) and AMK (11.8%). In *K. pneumoniae*, high resistance was observed against cefotaxime (86.4%), cefepime (85.4%), ceftazidime (85.4%), amoxicillin-clavulanic acid (85.4%) and gentamicin (70.0%), with low resistance level to meropenem (10.7%) and amikacin (21.3%). (Table 2).

**Multi-drug resistant Enterobacteriaceae**

Overall, 68.3% (291/426) of the *Enterobacteriaceae* isolates were multi-drug resistant (MDR, non-susceptible to at least 3 antibiotics belonging to different antibiotics categories), among which *E. coli* and *K. pneumoniae* contributed to 35.0% (150/426) and 20% (85/426) of the

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**Table 1** Distribution of *Enterobacteriaceae* isolates against demographic characteristics, specimen types and bacteriology laboratory, Addis Ababa, Ethiopia between Jan to May 2017

| Variables (Number) | Distribution Enterobacteriaceae isolate n (%) |
|--------------------|-----------------------------------------------|
| Gender             |                                               |
| Male (190)         | *E. coli* 90 (47.4)  | *K. pneumoniae* 54 (28.4)  |
| Female (236)       | *E. cloacae* 17 (8.9)  | *Citrobacter species* 17 (8.9)  |
|                    | *K. oxytoca* 5 (2.6)  | *K. ozaenae* 1 (0.5)  |
|                    | Other isolates 6 (3.1)  |
| Age group          |                                               |
| ≤ 28 days (24)     | *E. coli* 3 (12.5)  | *K. pneumoniae* 18 (75.0)  |
| 29 days- < 1 year (34) | *E. coli* 9 (26.5)  | *K. pneumoniae* 21 (61.8)  |
| 1- < 5 years (35)  | *E. coli* 16 (45.7)  | *K. pneumoniae* 9 (25.7)  |
| 5- < 15 years (42) | *E. coli* 6 (42.9)  | *K. pneumoniae* 4 (13.3)  |
| 15- < 25 years (35) | *E. coli* 14 (40.0)  | *K. pneumoniae* 5 (14.3)  |
| 25- < 65 years (190) | *E. coli* 119 (62.6) | *K. pneumoniae* 30 (15.8)  |
| > 65 years (66)    | *E. coli* 49 (74.2)  | *K. pneumoniae* 6 (9.1)  |
| Bacteriology laboratories |                                               |
| ICL (150)          | *E. coli* 109 (72.7) | *K. pneumoniae* 9 (6.0)  |
| EPHI (89)          | *E. coli* 36 (40.4)  | *K. pneumoniae* 29 (32.6)  |
| TASH (118)         | *E. coli* 53 (44.9)  | *K. pneumoniae* 33 (28.0)  |
| YHMC (69)          | *E. coli* 30 (43.5)  | *K. pneumoniae* 32 (46.4)  |
| Types of Specimen  |                                               |
| Urine (272)        | *E. coli* 188 (69.1) | *K. pneumoniae* 32 (11.8)  |
| Blood (90)         | *E. coli* 24 (26.7)  | *K. pneumoniae* 53 (58.9)  |
| Pus (40)           | *E. coli* 8 (20.0)  | *K. pneumoniae* 12 (30)  |
| Sputum (6)         | *E. coli* 1 (16.7)  | *K. pneumoniae* 2 (33.3)  |
| CSF (2)            | *E. coli* 0 (0.0)  | *K. pneumoniae* 2 (100.0)  |
| Body fluids (11)   | *E. coli* 5 (45.5)  | *K. pneumoniae* 2 (18.2)  |
| Ear & Eye discharge(5) | *E. coli* 2 (40.0)  | *K. pneumoniae* 0 (0.0)  |
| Total (N = 426)    | *E. coli* 228 (53.5) | *K. pneumoniae* 103 (24.1)  |

*Other isolates are P. mirabilis, Providencia species, M. morganii and E. aerogenes*
observed MRD, respectively. We found that the highest MDR level was observed among *K. pneumoniae* isolates (83.5%, 86/103) followed by *citrobacter species* (68.9%, 20/29), *E. coli* (66.2%, 151/228), and *E. cloacae* (63.6%, 14/22). None of *P. mirabilis* was found to be MDR. Only 11.3% (48/426) of the determined enterobacteriaceae were susceptible for all antibiotics tested in this study (Table 3). From all MDR *Enterobacteriaceae*, the predominant were *E. coli* (51.9%; 151/291) and *K. pneumoniae* (29.6%; 86/291) (Fig. 1).

**Table 2** Distribution of antibiotics resistance among *Enterobacteriaceae* isolates, Addis Ababa, Ethiopia between Jan to May 2017

| Isolates (number) | Distribution of antibiotics resistance among *Enterobacteriaceae* isolates (n (%)) |
|-------------------|----------------------------------------------------------------------------------|
|                   | CTX CAZ CFP FOX MER SXT CPR GEN AMK AMC NOR^a N/Total                           |
| E. coli (n = 228)  | 125 (54.8) 121 (53.1) 122 (53.5) 50 (21.9) 8 (3.5) 177 (77.6) 146 (64.0) 76 (33.3) 27 (11.8) 160 (70.0) 121/188 |
| K. pneumoniae (n = 103) | 89 (86.4) 88 (85.4) 88 (85.4) 24 (23.3) 11 (10.7) 89 (86.4) 52 (50.5) 72 (70.0) 22 (21.3) 87 (85.4) 18/32 (56.2) |
| E. aerogens (n = 2) | 1 (50) 0 (0) 1 (50) 0 (0) 1 (50) 0 (0) 1 (50) 0 (0) 1 (50) 0 (0) |
| M. morganii (n = 5) | 1 (20) 0 (0) 1 (20) 0 (0) 1 (20) 0 (0) 1 (20) 0 (0) 1 (20) 0 (0) |
| P. mirabilis (n = 7) | 3 (42.8) 3 (42.8) 3 (42.8) 2 (28.6) 0 (0) 3 (42.8) 2 (28.6) 1 (14.3) 0 (0) 4 (57.1) 2/4 (50.0) |
| M. morganii (n = 13) | 7 (53.8) 7 (53.8) 6 (46.2) 2 (15.4) 0 (0) 8 (61.5) 6 (46.2) 5 (38.5) 0 (0) 8 (61.5) 0/5 (0.0) |
| Citrobacter. spps (n = 10) | 5 (50.0) 5 (50.0) 5 (50.0) 7 (70.0) 1 (10) 7 (70.0) 5 (50.0) 3 (30.0) 0 (0) 5 (50.0) 5/7 (71.4) |
| Providencia spps (n = 7) | 3 (42.8) 3 (42.8) 3 (42.8) 2 (28.6) 0 (0) 3 (42.8) 2 (28.6) 1 (14.3) 0 (0) 4 (57.1) 2/4 (50.0) |
| P. mirabilis (n = 5) | 1 (20) 1 (20) 1 (20) 0 (0) 0 (0) 3 (60) 2 (40) 1 (20) 0 (0) 0 (0) 0/1 (0) |
| M. morganii (n = 2) | 2 (100) 2 (100) 2 (100) 1 (50) 0 (0) 2 (100) 1 (50) 1 (50) 0 (0) 2 (100) 0 (0) |
| E. aerogens (n = 2) | 1 (50) 1 (50) 1 (50) 0 (0) 0 (0) 1 (50) 0 (50) 1 (50) 0 (0) 1 (50) 0 (0) 0/2 (0) |
| Total Resistance (N = 426) | 265 (62.2) 257 (60.8) 259 (60.3) 107 (25.1) 426 (100) 324 (77.0) 240 (46.3) 185 (43.4) 89 (86.4) 160/272 (58.8) |

Abbreviations: CTX ceftaxime, CAZ cefazidime, FOX cefoxitin, CFP ceferpine, MER meropenem, CPR ciprofloxacin, NOR norfloxacin, SXT sulfamethoxazole-trimethoprim, GEN gentamycin, AMK amikacin, AMC amoxicillin with calvulanic acid

^aNorfloxacin antibiotics disks were used for isolates from urine specimen

**Magnitude of ESBL producing Enterobacteriaceae**

Of all the *Enterobacteriaceae* isolates, 62.2% (265/426) were positive for the screening test of ESBL production as measured with ceftaxime zone of inhibition ≤27 mm and ceftaxime zone of inhibition ≤22 mm. Using the combination disk test, we confirmed that 92.8% (246/265) of the suspected isolates were able to produce ESBL resulting in an overall ESBLs positivity of 57.7% (246/426) (Table 4). From all the isolate, *E. coli* accounted 27.9% (119/426), *K. pneumoniae* 19.0%

**Table 3** Multidrug resistance level of *Enterobacteriaceae* to different classes of antibiotics, Addis Ababa, Ethiopia between Jan to May 2017

| Isolates (number) | Level of antibiotics resistance (n (%)) |
|-------------------|----------------------------------------|
|                   | RO R1 R2 R3 R4 R5 R6 R7 Total MDR-E (>R3) |
| E.coli (228)      | 20 (8.8) 28 (12.3) 29 (12.7) 34 (14.9) 44 (19.3) 50 (21.9) 16 (7.0) 5 (2.2) 149 (65.3) |
| K.pneumoniae (103) | 6 (5.8) 5 (4.8) 7 (6.8) 10 (9.7) 22 (21.3) 33 (32.0) 11 (10.7) 9 (8.7) 85 (82.5) |
| E.cloacae (22)    | 4 (18.2) 2 (9.1) 2 (9.1) 3 (13.6) 2 (9.1) 3 (13.6) 6 (27.3) 0 (0) 14 (63.6) |
| C.diversus (19)   | 3 (15.8) 2 (10.5) 1 (5.3) 1 (5.3) 3 (15.8) 5 (26.3) 3 (15.8) 1 (5.3) 13 (68.4) |
| K.oxytoca (15)    | 5 (33.3) 2 (13.3) 1 (6.7) 0 (0) 2 (13.3) 4 (26.7) 0 (0) 1 (6.7) 7 (46.6) |
| K.ozaenae (13)    | 1 (7.7) 3 (23.1) 1 (7.7) 2 (15.4) 5 (38.5) 1 (7.7) 0 (0) 0 (0) 8 (61.5) |
| Citrobacter. Spps(10) | 3 (30.0) 0 (0) 0 (0) 3 (30.0) 0 (0) 0 (0) 2 (20.0) 1 (10.0) 1 (10.0) 7 (70) |
| Providencia Spps(7) | 3 (42.8) 0 (0) 1 (24.3) 0 (0) 2 (28.5) 1 (24.3) 0 (0) 0 (0) 3 (42.8) |
| P. mirabilis (5)  | 2 (40.0) 1 (20.0) 2 (20.0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) |
| M. morganii (2)   | 0 (0) 0 (0) 0 (0) 1 (50) 0 (0) 0 (0) 1 (50) 0 (0) 0 (0) 0 (0) 0 (0) |
| Total(N = 426)    | 48 (11.3) 43 (10.1) 43 (10.1) 54 (12.7) 81 (19.0) 101 (23.7) 38 (8.9) 17 (4.0) 291 (68.3) |

Abbreviations: RO stands for resistance for zero antibiotics; R1 stands for resistance to one drug, R2 stands for resistance to two drugs and so on; and ≥R3 stands for resistance to 3 or more antibiotics from different classes; MDR-E stands for multi-drug resistant *Enterobacteriaceae*
The distribution of ESBL producers varied among different species of Enterobacteriaceae. The highest intra-species frequency of ESBL production was observed among K. pneumoniae 78.6% (81/103) followed by E. coli and Citrobacter species with 52.2% (119/228) and 51.7% (15/29), respectively (Fig. 2). The lowest intra-species ESBL production was observed in P. mirabilis with 20% (1/5) proportion.

Regarding ESBL-producing Enterobacteriaceae distribution across age groups, a higher proportion was observed among isolates from patients less than 1 year (86.2%), < 28 days (87.5%), and 5 to < 15 years (69.0%) compared with other age groups. The total proportion of ESBL-producing Enterobacteriaceae among children < 15 years was 74.1% (100/135).

**Distribution of MDR and ESBL-producing Enterobacteriaceae**

The magnitude of ESBL-producing Enterobacteriaceae was different in the four microbiology laboratories. The magnitude was highest in TASH (71.5%; 84/118) followed by YHMC (68.1%; 47/69) and EPHI (66.3%; 59/89), and lowest in ICL (37.3%; 56/150). In all laboratories, the highest ESBL production was observed among K. pneumoniae (78.6%; 81/103). Distribution of MDR Enterobacteriaceae and major ESBL-producing Enterobacteriaceae at the four microbiology laboratories is presented in Table 4.

**Distribution of ESBL-producing Enterobacteriaceae with their MDR level among different clinical specimens**

From all specimens included in this study, the highest magnitude of ESBL-producing Enterobacteriaceae (84.4%; 76/90) and MDR (83.3%; 75/90) was found in blood. In the urine specimen, the extent of ESBL-producing Enterobacteriaceae and MDR were 50.7% (138/272) and 66.5% (181/272), respectively (Table 5). Of all ESBL-producing Enterobacteriaceae, 96.3% (237/246) were MDR, whereas only 30% (54/180) of the non–ESBL producers were MDR. There was a significant correlation (Pearson correlation of 0.759, p-value of 0.01) between ESBL production and MDR Enterobacteriaceae. Binary logistic regression or bivariate analysis also showed that being an ESBL producer has statistically significant association with MDR (P < 0.001).

That is, the odds of being MDR were 61.4 times (95% CI
COR = 29.37 to 128.53) more likely among ESBL-producing Enterobacteriaceae than non-ESBL isolates.

**Antibiotics susceptibility pattern of ESBLs-E to potentially active drugs**

The most active drugs for ESBL-producing isolates were meropenem, amikacin, and cefoxitin, with susceptibility results of 96.7, 82.1, and 70%, respectively. Moreover, 37, 29, and 10.2% of ESBL-producing isolates were susceptible to gentamicin, ciprofloxacin, and cotrimoxazole, respectively. Non-ESBL-suspicious isolates were 100, 96.3, and 91.3% sensitive to meropenem, amikacin, and cefoxitin, respectively. Furthermore, gentamicin and ciprofloxacin remained active against 90.1 and 70.2% respectively of non-ESBL-suspicious Enterobacteriaceae. The antibiotic susceptibility of ESBL confirmatory test positive, ESBL screening test positive-producing, and non-ESBL-suspicious (screening negative) Enterobacteriaceae is displayed in Fig. 3.

**Discussions**

ESBL-producing Enterobacteriaceae have become a serious worldwide problem. Dissemination of ESBLs compromises the activity of broad-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcomes for patients [19].

**Prevalence of ESBL-producing Enterobacteriaceae**

In the present study, the magnitude of ESBL-producing Enterobacteriaceae was 57.7%, which is higher than magnitudes reported by previous researchers in Ethiopia: 38.4% in Jimma by Siraj and his colleagues [20], 36% in Jimma by Mulualem Y and his colleagues [21], 33.3% in Harar [22] and 25% in Adama [23]. The emergence of ESBL-producing Enterobacteriaceae in higher magnitude in Addis Ababa emphasizes the need to implement strong infection control strategies.

The magnitude of ESBL-producing Enterobacteriaceae (57.7%) in our study was comparable with a studies in Bahir-Dar-Ethiopia (57.6%) [24], Burkina Faso (58.0%) [25], Uganda (62.0%) [26], Ghana (49.3%) [27], and Karnataka-India (57.5%) [28]. One of the most important factors in the emergence of ESBLs production is the selective pressure caused by the use of 3rd generation cephalosporins [29, 30]. Lack of antibiotic surveillance, antibiotics misuse, and weak infection control measures may also contribute to the high magnitude of ESBL.

Compared with the present study, ESBL-producing Enterobacteriaceae prevalence in Europe is lower; 0.7% in Austria and 23.8% in Turkey [31], and 6.3% in Italy [32]. The difference might be due to infection control strategies in those countries. Moreover, our finding is higher than levels seen in some non-European countries,
such as Egypt (16%) [33] and Nepal (24.4%) [34]. The difference may be due to the study participant and method difference.

The predominant ESBL-producing isolates in this study, *K. pneumoniae* (78.6%) and *E. coli* (52.2%) were in agreement with studies done in Bahir-Dar, Ethiopia: (*K. pneumoniae* 69.8%, *E. coli* 58.2%) [24], Jimma, Ethiopia (*K. pneumoniae* 70.4%, *E. coli* 28.2%) [20], and Uganda: (*K. pneumoniae* 72.7%, *E. coli* 58.1) [26]. However, *E. coli* was a predominant ESBL producer compared with *K. pneumoniae* in studies in Adama, Ethiopia (*E. coli* 51.5%, *K. pneumoniae* 11.5%) [23], Burkina Faso (*E. coli* 67.5%, *K. pneumoniae* 26%) [25], India (*E. coli* 61.4%, *K. pneumoniae* 46.2%) [28] and Central India (*E. coli* 50.14%, *K. pneumoniae* 48.27%) [35].

The proportion of ESBL-producing *Enterobacteriaceae* among children under 15 years (74.1%) was in agreement with the previous studies done in Addis Ababa TASH (78.57%) [36], Tertiary Care Hospital of North-West India (66.7%) [37] and in rural Ghana (68%) [38] [38]. However, our finding was higher compared to a study conducted in Burkina Faso (50.8%) [25].

**Distribution of ESBL-producing Entrobacteriaceae among different specimens**

In our study ESBL-producing *Enterobacteriaceae* were found predominantly in blood specimens (84.4%, 76/90) followed by wound/pus specimens (52.5%, 21/40), urine (50.7, 138/272) and other specimens (CSF & other body fluids, sputum, ear and eye discharge) (45.8%, 11/24). Other investigator also reported blood as a major source of ESBL-producers in Bahir-Dar Dar (84.8% in blood, 72.7% in open wound swabs) [24], Burkina Faso (75% in blood) [25], Iran (87.8% in blood, 48.5% in urine) [39], North West India (79.2.0% in blood) [37] and again in India (66.67% in blood, 54.67% in urine) [40]. This indicates that ESBL-producing *Enterobacteriaceae* are becoming a serious problem in the treatment of invasive bacterial infections. However, in other studies urine was the major source of ESBL-producers: central India (52.28% in urine) [35], Uganda (64.9% in urine, 47.4% in pus) [26], Bangladesh (70.4% in urine, 16.5% in blood) [41]. The difference might be attributed to the difference in the study participants, risk factors or extent of antibiotics use.

**Antibiotics susceptibility pattern of ESBL-producing *Enterobacteriaceae***

In this study, ESBL-producing isolates were found to be susceptible primarily to meropenem (96.7%), amikacin (82.1%), and cefoxitin (70%). This was in close agreement with studies done in Ghana (meropenem 100%) [27], central India (meropenem 87.5%, amikacin 83.92%) [35], Jimma, Ethiopia (amikacin 83.7%) [20], and India (meropenem 94.0%, amikacin 82.6%) [42]. The results indicate that these antibiotics were the most active treatment of choice for ESBL-producing *Enterobacteriaceae*.

The present study, the levels of co-resistance within different classes of antibiotics among the ESBL-producing *Enterobacteriaceae* were significantly higher for most antibiotics tested. Of ESBL-producers, 63% were non-susceptible to gentamicin, 89.8% to trimethoprim-sulfamethoxazole, 69% to ciprofloxacin, 97.6% to cefepime, and 91.5% to amoxicillin-clavulanic acid. Our finding is comparable with the study conducted in Israel, which showed that 75% of ESBL-producer isolates were non-susceptible to gentamicin, 70% to trimethoprim-sulfamethoxazole and 59% to ciprofloxacin [9], and also comparable with studies in
Burkina Faso (45% to trimethoprim-sulfamethoxazole, 89% to gentamicin, 80% to ciprofloxacin) [25], Ghana (92.6% to trimethoprim-sulfamethoxazole, 91.2% to gentamicin, 41.1% to ciprofloxacin) [27], Nepal (90.7% to ciprofloxacin, 90.4% to trimethoprim-sulfamethoxazole, 63.12% to gentamicin) [34], and central India (50% to gentamicin, 87.5% to ciprofloxacin, 94.6% to trimethoprim-sulfamethoxazole) [25]. These findings indicate that ESBL-producing Enterobacteriaceae were the major cause of resistance to various antibiotics classes, as these bacteria are typically nosocomial.

Antibiotics resistance pattern among all Enterobacteriaceae isolates
In the present study, high resistance was observed to sulfamethoxazole-trimethoprim (77.0%) followed by amoxicillin with clavulanic acid (71.6%), cefotaxime (62.2%), ceftazidime (60.8%), cefepime (60.3%), norfloxacin (58.8%), ciprofloxacin (46.3%) and gentamycin (43.4%). The results of our study are in line with the findings of studies conducted in Iran (sulfamethoxazole-trimethoprim 92.8%) [48], Bahir-Dar (93.1%) [53], Nepal (96.84%) [43], and Sierra Leone (85.7%) [44]. The difference in magnitude of MDR isolates might be due to the selection of antibiotic from a different class, the definition for MDR, study period and specimen type, and the difference in the study population.

There was an intra-species difference in MDR level. The present study showed that the level of MDR in K. pneumoniae (82.5%) and E. coli (65.3%) was comparable with studies conducted in Equatorial Guinea (E. coli 74.4%) [49], Sierra Leone (K. pneumoniae 73.3%, E. coli 61.5%) [44]. However, our result is lower than studies conducted in Gondar, Ethiopia (K. pneumoniae 95.6%, E. coli 92.9%) [52], Khartoum, Sudan (E. coli 92.2%) [48], and Equatorial Guinea (K. pneumoniae 91.7%) [49]. The MDR level among E. coli (50.2%) in Dessie, Ethiopia is lower than our study [45]. The difference in MDR level among K. pneumoniae and E. coli in our study might be due to most K. pneumoniae being isolated from blood specimens collected from hospital inpatients.

In this study 237 (96.3%) of the ESBL-producers were MDR strains, whereas only 54 (30%) of the non-ESBL-producers were MDR strains. The ESBL-producing isolates had increased resistance compared with non-ESBL-producers indicating that MDR is expected to be more common in ESBL-producing bacteria.

Multi-drug resistance among Enterobacteriaceae
In the present study, the overall magnitude of MDR among all Enterobacteriaceae isolate (68.3%) was fairly similar with a study done in Dessie, Ethiopia (74.6%) [45], and Equatorial Guinea (93.5 and 87.4%) [46, 52]. The higher proportion of MDR limits the treatment option for hospital-acquired infections caused by Enterobacteriaceae. On the other hand, our result was lower than findings from other studies in Gondar, Ethiopia (93.5 and 87.4%) [46, 52], Bahir-Dar (93.1%) [53], Nepal (96.84%) [43], and Sierra Leone (85.7%) [44]. The difference in magnitude of MDR isolates might be due to the misuse or overuse of the antibiotics coupled with weak infection control measures [19]. The high resistance rate of K. pneumoniae alerts the health care system to work hard on the health facilities infection control.

Strength of the study
This is the first study done at multiple health facilities on the magnitude of ESBL-producing Enterobacteriaceae in Addis Ababa, Ethiopia. This multi-centered study can reveal the extent of distribution of ESBLs and MDR among Enterobacteriaceae and the degree of resistance to other non-beta-lactam antibiotics. The magnitude of ESBLs and MDR in the city was done in a relatively larger number of specimens and isolates than in earlier studies.
Limitation of the study

- Although combinations of aminoglycosides and fluoroquinolones were tested, other beta-lactams and beta-lactamase inhibitors, such as ticarcilin, colistin, and piperacillin/tazobactam, were not tested, as they were beyond the scope of this study.
- We are unable to see possible risk factors, certain clinical features and the outcome of the patients infected with ESBL-producing or MDR bacteria, due to lack of adequate resource.
- Although most of the study isolates were collected from inpatients, the exact number of nosocomial versus community-acquired bacteria were not differentiated.
- The isolates were collected from four bacteriology laboratories in Addis Ababa, but the results may not be applied to the entire city or country.

Conclusion and recommendation

There was a high prevalence of ESBL-producing Enterobacteriaceae and MDR isolates. The majority of ESBL-producing isolates were found primarily in blood and urine specimens. The most frequent ESBL-producing Enterobacteriaceae were K. pneumoniae and E. coli. A higher level of resistance to multiple classes of antibiotics was observed among ESBL producers compared with non-ESBL producers. The better options for the treatment of ESBL-producing Enterobacteriaceae are meropenem, amikacin, and cefotixin. ESBL-producing isolates showed a high rate of resistance to ciprofloxacin, cefepime, cotrimoxazole, and gentamicin compared with non-ESBL producers. The rise of MDR and ESBLs necessitates the strengthening of clinical bacteriology research and the diagnostic capacity of laboratory professionals for the detection and surveillance of antibiotic resistance. We recommend routine screening of ESBLs production of Enterobacteriaceae along with strong infection prevention strategies.

Abbreviations

CDT: Combination disk test; CLSI: Clinical and Laboratory Standards Institute; EPHI: Ethiopian Public Health Institute; ESBL-E: Extended-spectrum beta-lactamases Enterobacteriaceae; ESBLs: Extended-spectrum beta-lactamases; ICL: International Clinical Laboratories; MDR: Multidrug Resistant; TASH: Tikur Anbessa Specialized Hospital; YHMC: Yekatit 12 Hospital Medical College

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Availability of data and materials

The current study data sets used for analysis can be obtained from the corresponding author through email (dejenie21@gmail.com) on reasonable request.

Authors’ contributions

DS: Conceived, designed, analyzed and interpreted the research; and also wrote the manuscript. TL, AA and HK: Participated in the technical laboratory works and data collection. KD and MH: Supervised the study through their critical review of the research and the manuscript write up. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study proposal was reviewed and approved by the department of research and ethics review committee of the Medical Laboratory sciences, College of Health Sciences; Addis Ababa University (Ref. No. MLS/223/17). Permission was obtained from the respective laboratories in where the isolate and data were collected.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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