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Polyclonal spread of $bla_{CTX\text{-}M\text{-}15}$ through high-risk clones of *Escherichia coli* at a tertiary hospital in Ethiopia

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ABSTRACT

Objectives: The burden of antimicrobial resistance and spread of epidemic clones are rarely reported from low-income countries. We aimed to investigate the genome-based epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) at a tertiary hospital in Jimma, Ethiopia.

Methods: Bacteria were isolated from clinical specimens at Jimma Medical Center and subjected to species identification (MALDI-TOF), antimicrobial susceptibility testing (disk diffusion) and whole-genome sequencing (Illumina, HiSeq2500). Genomic data analysis was performed using EnteroBase and Center for Genomic Epidemiology bioinformatics pipelines. A maximum likelihood tree was generated using FastTree/2.1.8 based on single nucleotide polymorphisms (SNPs) in shared genomic regions to identify transmission clusters.

Results: *Escherichia coli* isolates (n = 261) were collected from 1087 single non-duplicate clinical specimens over a 5-month period in 2016. The prevalence of ESBL-EC was 54.8% (143/261), 96% of which were resistant to multiple antibiotic classes. The $bla_{CTX\text{-}M\text{-}15}$ ESBL gene was present in 88.4.% of isolates (122/138). Genes conferring resistance to aminoglycosides and ciprofloxacin [*aac(6′)-Ib*-cr, 62.3% (86/138)], phenicols [*catB3*, 56.5% (78/138)], sulfonamides [*sul1*, 68.1% (94/138), trimethoprim [*dfrA17*, 58.0% (80/138)] and macrolides [*mph(A)*, 67.4% (93/138) were detected. The most prevalent sequence types were ST410 (23%), ST648 (17%), ST131 (10%) and ST167 (7%). Isolates of the same sequence type collected from different units of the hospital were highly similar in the SNP analysis.

Conclusion: A high prevalence of ESBLs and dissemination of $bla_{CTX\text{-}M\text{-}15}$ through multiple high-risk *E. coli* clones was detected. Nosocomial spread of multidrug-resistant ESBL-EC within the hospital puts vulnerable patients at risk of difficult-to-treat infections.

1. Introduction

Antimicrobial resistance has become a major concern worldwide. Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) continue to contribute to the increasing burden of antimicrobial resistance. In-depth epidemiological studies of high-risk clones, including sequence types ST131, ST410, ST648 and ST405, are important to understand the global spread of the problem [1–3]. However, such studies are scarce from low- and middle-income countries.

Multidrug-resistant *E. coli* strains are continuously reported from different parts of the world. Although the prevalence of ESBL-EC is relatively low in the USA and Northern Europe, it is currently increasing [4,5]. An increase in the prevalence of ESBL-EC isolates has also been reported from Asian countries. A recent re-

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view showed that the prevalence of bla_{CTX-M} genes reached 60–79% among clinical isolates of ESBL-EC in Asia [6]. Moreover, several reports from different parts of the world show that the high prevalence of bla_{CTX-M}-mediated antibiotic resistance may be spreading worldwide [7–9].

In Africa, an increasing trend in the prevalence of ESBL-producing bacteria has been demonstrated via phenotypic studies. A study from Mali in 2017 showed that the prevalence of bla_{CTX-M} genes was 79% among clinical *E. coli* isolates [10]. Several previous phenotypic studies conducted in Ethiopia reported a high prevalence of ESBL-EC from different hospitals (62.5% from Jimma [11] and 78.5% [12] and 57.7% [13] from Addis Ababa). High-risk clones of *E. coli* are another global problem; however, this has not been well studied in African countries. Deeper genomic studies are limited and many of the genetic studies conducted on ESBL-EC had a small sample size [11,14,15].

Here we aimed to determine the prevalence of ESBLs, additional antimicrobial resistance genes, pathotypes and epidemiological clones of *E. coli* isolated at Jimma Medical Center in Jimma, Ethiopia. Since detecting nosocomial transmission is important to ensure patient safety and to guide the implementation of efficient infection control measures, we compared isolates from different units of the hospital.

2. Materials and methods

2.1. Study design and population

We performed a cross-sectional study among patients seeking medical care at Jimma Medical Center, a 600-bed hospital serving a total population of 15 million. Non-duplicate single specimens were collected from patients with suspected infections (pneumonia, diarrhoea, wound infection and urinary tract infection) from June–October 2016. Data regarding sociodemographic variables as well as possible risk factors including clinical diagnosis, presence of underlying chronic illnesses, whether the patient is admitted to the hospital, and current usage of antibiotics, were collected using a structured questionnaire. Bacteria were primarily isolated and identified by conventional biochemical tests, and the ESBL phenotype was confirmed by double disk synergy test using cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg) and amoxicillin/clavulanic acid (20/10 μg). Species identification was further confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass (MALDI-TOF), and antimicrobial susceptibility testing was performed for piperacillin/tazobactam (30/6 μg), cefotaxime (5 μg), ceftriaxone (30 μg), ceftazidime (10 μg), meropenem (10 μg), ertapenem (10 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg) and trimethoprim/sulfamethoxazole (25 μg) by the Kirby–Bauer disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (https://www.eucast.org/ast_of_bacteria/).

2.2. DNA extraction and whole-genome sequencing (WGS)

All *E. coli* isolates phenotypically confirmed as ESBL-producers were selected for WGS. After overnight aerobic incubation on cysteine–lactose–electrolyte-deficient (CLED) agar at 37°C, two to five pure colonies were taken with an inoculating loop and suspended in bovine serum albumin (0.5 mg/mL) in phosphate-buffered saline (pH 7.4). Cell suspensions were then dispensed into a 96-well plate and genomic DNA was extracted in an automated DNA extraction system (MagNA Pure 96; Roche Life Sciences, Sweden). The concentration of DNA was measured with a Qubit 3.0 fluorometer (Thermo Fisher Scientific). Libraries were generated using a Nextera XT Kit (Illumina Inc.), and WGS was performed on an Illumina HiSeq 2500 system (Illumina Inc.) with a 150-bp insert size paired-end sequencing protocol at the Science for Life Laboratory (Stockholm, Sweden).

2.3. Genomic assembly and genomic data analysis

SPAdes v.3.9 was used for genome assembly. The assembled draft genomes were used for extraction of resistome, virulome, plasmid and serotyping of the strains using Center for Genomic Epidemiology (CGE) tools (http://www.genomicepidemiology.org/). EnteroBase (http://enterobase.warwick.ac.uk) was used for analysis of multilocus sequence typing (MLST) sequence types (STs) and identification of population structures. All putative genes/genomic traits were considered positive only when thequery match the reference with 100% identity in coverage and nucleotide similarity..

Variant calling, mapping and de novo assembly were done using CLC Assembly Cell v.4.4.2.133896 (Qiagen Bioinformatics). Minimum coverage was set to 10 and the single nucleotide polymorphism (SNP) ratio cut-off for SNP support was set to 90%. Gubbins v.2.3.1, with standard settings was used to remove SNPs from recombinant regions [16]. A maximum likelihood tree was inferred with FastTree/2.1.8 [17] using a general time reversible (GTR) nucleotide substitution model and bootstrapping (1000 replicates). Visualisation of trees and metadata was done using iTOL (https://itol.embl.de/; accessed 2019-03-05) [18]. Univariate descriptive statistical analysis was performed using IBM SPSS Statistics v.20.0 (IBM Corp., Armonk, NY, USA). A χ^2 test *P*-value of $\leq$0.05 was applied as the cut-off for statistical significance.

2.4. Ethics and data availability

The study was conducted after receiving ethical approval from institutional and national level review boards. Moreover, the study was conducted after written consent was obtained from the study participants, and the findings of each clinical sample were communicated to the physician in charge of each patient's care. The genomic sequences were submitted to NCBI (BioProject accession no. **PRJNA593604**).

3. Results

3.1. Prevalence of extended-spectrum β-lactamases (ESBLs) and patient characteristics

We enrolled a total of 1087 patients with suspected infections at Jimma Medical Center in 2016. Recruitment of patients was done at four main units of the hospital, namely the intensive care unit (ICU) (n = 42), medical unit (n = 509), surgical unit (n = 361) and paediatric unit (n = 175). The type and number of specimens collected were as follows: urine (n = 456); sputum (n = 267); stool (n = 180); wound swab (n = 181); and cerebrospinal fluid (CSF) (n = 3).

Overall, 62.9% (684/1087) of patients were reportedly on antimicrobial therapy and 39.9% (427/1087) were currently on ceftriaxone treatment as monotherapy or combined with other drugs. Penicillins (19.5%; 212/1087), metronidazole (11.8%; 128/1087), chloramphenicol (5.9%; 64/1087), aminoglycosides (3.9%; 42/1087) and fluoroquinolones (2.8%; 20/1087) were the most common antimicrobials prescribed at the hospital.

A total of 642 bacterial isolates belonging to several species were isolated from the clinical samples (urine, diarrhoeic stool, sputum, wound swab and CSF). The overall prevalence of ESBL-producers from all bacterial species isolated was 60.1% (386/642). *Klebsiella pneumoniae, Enterobacter* spp. and *E. coli* were the most common Enterobacteriaceae species, and *E. coli* (40.7%; 261/642)

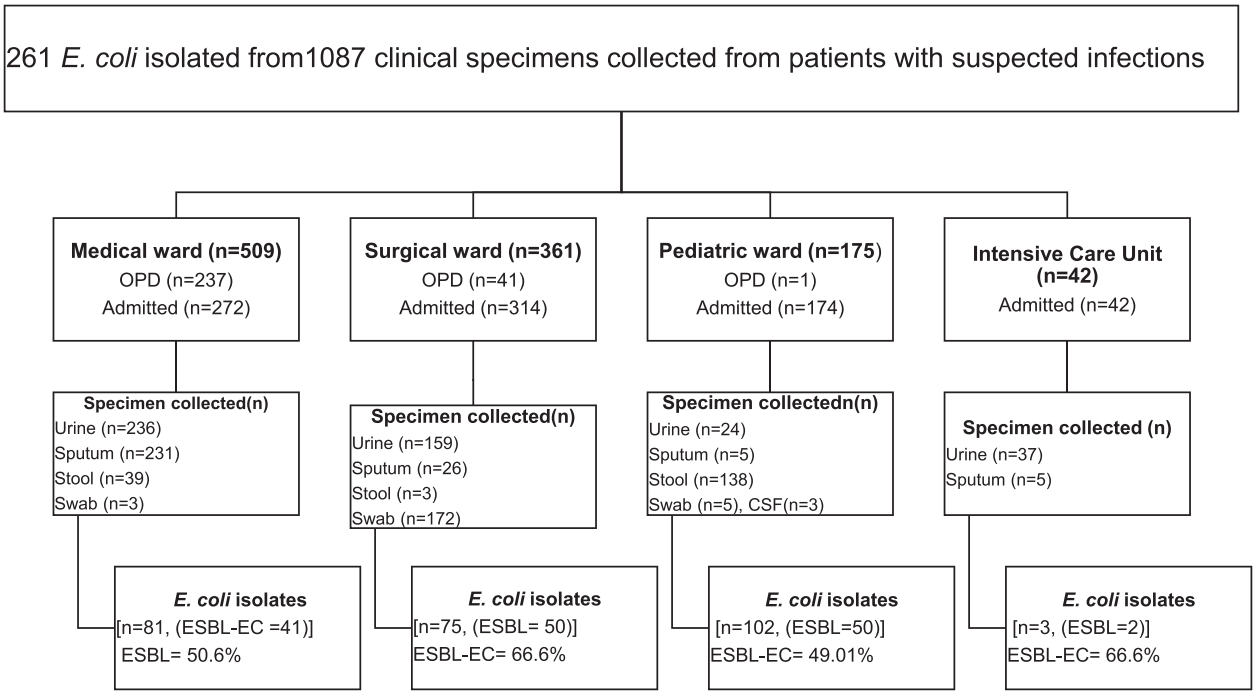

Fig. 1. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) isolated from patients with suspected infection at Jimma Medical Center, Ethiopia. OPD, outpatient department; ICU, intensive care unit.

was the most prevalent species. The prevalence of ESBL-producers vary between species: *Klebsiella* spp., 76.7% (112/146); *Enterobacter* spp., 69.4% (43/62); and *E. coli*, 54.8% (143/261) (Table 1). A higher prevalence of ESBL-producing *E. coli* isolates was detected in the surgical ward (66.7%; 50/75) compared with other wards at the hospital [ICU (66.7%, 2/3); medical unit (50.6%; 41/81); and paediatric unit (49.0%; 50/102)] (Fig. 1; Table 1).

Possible risk factors for the overall increased prevalence of ESBL-producing Gram-negative isolates were investigated. Among these factors, age <5 years, admission to the hospital, paediatric ward and presence of underlying chronic illness showed statistically significant associations with a higher prevalence of ESBL-producing Gram-negative isolates. Sex and current use of antibiotics (reported use of antibiotics) showed no statistically significant association (Table 2). More details of the data regarding admission, sample type, sequence type (ST), pathotype, phylotypes, FimH types and serotype of the ESBL-EC strains are summarised in Supplementary Table S1.

3.2. Extended-spectrum β-lactamase (ESBL) genotypes and the overall resistomes

The most prevalent ESBL genotype was $bla_{\text{CTX-M-15}}$ (88.4%; 122/138), and other β-lactam resistance genes identified included $bla_{\text{OXA-1}}$ (63.0%; 87/138), $bla_{\text{TEM-1B}}$ (54.3%; 75/138) and $bla_{\text{CMY-2}}$/$bla_{\text{CMY-42}}$ (13.8%; 19/138) (Table 3). All of the bla_{CMY} genes occurred together with the $bla_{\text{CTX-M-15}}$ ESBL gene. The $bla_{\text{SHV-12}}$ ESBL gene ($n = 1$) was detected in a single isolate. Several non-β-lactam antimicrobial resistance genes were also identified with the $bla_{\text{CTX-M-15}}$ gene, including resistance genes to aminoglycosides and fluoroquinolones [multiple genes were detected for aminoglycosides, but *aac(6′)-Ib*-cr was the most prevalent (62.3%; 86/138)], phenicols [*catB3* (57.2%; 79/138)], sulfonamides [*sul1* (68.8%; 95/138)], trimethoprim [*dfrA17* (58.7%; 81/138)], macrolides [*mph(A)* (67.4%; 93/138)] and tetracycline [*tetB* (44.9%; 62/138)]. The fosfomycin resistance gene *fosA* ($n = 1$) and the rifampicin resistance gene *arr-3* ($n = 3$) were rarely identified in this study. Genes encoding resistance to multiple classes of antimicrobials were observed; the resistome matrix is given in Supplementary Table S2.

Antimicrobial susceptibility testing showed that the ESBL-EC isolates were resistant to multiple classes of antimicrobials with an overall combined resistance of 96% for at least three classes of antibiotics (Supplementary Table S3). These combined resistances include ESBL + piperacillin/tazobactam (86.2%; 119/138), ESBL + ertapenem (19.6%; 27/138), ESBL + meropenem (11.6%; 16/138), ESBL + ciprofloxacin (88.4%; 122/138), ESBL + gentamicin (60.1%; 83/138) and ESBL + sulfamethoxazole/trimethoprim (94.2%; 130/138). The low prevalence of resistance to meropenem and ertapenem is important to consider at this hospital.

3.3. Multilocus sequence typing (MLST) and pathotypes

Based on the Clermont phylotyping method [19], the ESBL-EC isolates were classified into the following phylogroups: A (33.3%; 46/138); B1 (8.7%; 12/138); B2 (6.5%; 9/138); C (18.1%; 25/138); D (17.4%; 24/138); E (5.1%; 7/138); and F (10.9%; 15/138). Pathotype classification was performed based on the profile of pathotype-defining virulence genes present in the isolates as described in a previous review [20]. The isolates were classified as enteropathogenic *E. coli* (EPEC) (46.4%; 64/138), extraintestinal pathogenic *E. coli*/uropathogenic *E. coli* (ExPEC/UPEC) (32.6%; 45/138), enteroaggregative *E. coli* (EAEC) (13.0%; 18/138), enterotoxigenic *E. coli* (ETEC) (2.9%; 4/138) and other diarrhoeagenic *E. coli* (DEC) (5.1%; 7/138). The most prevalent serotypes included O8 (27.5%; 38/138), O89 (13.8%; 19/138), O25 (6.5%; 9/138), O9 (6.5%; 9/138) and O102 (5.8%; 8/138) (Supplementary Table S1).

More than 30 different sequence types (STs) were identified, with ST410 (15.2%; 21/138), ST648 (10.9%; 15/138), ST10 (7.2%; 10/138), ST131 (5.8%; 8/138), ST2659 (5.8%; 8/138) and ST167 (5.8%; 8/138) being the most prevalent (Supplementary Table S4). Moreover, 75% (6/8) of the ST131 isolates were O25:H4-ST131-*H*30-Rx/C2, which is a pandemic clone [3]. Core genome MLST based phylogenetic analysis showed that these isolates clustered with a

Table 1
Extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria isolated at different units in the hospital from June–October 2016

Species	Hospital ward in which the strains were isolated								
	Medical		Surgical		Paediatric		ICU		Total	
	No. of isolates	No. (%) of ESBL-producers	No. of isolates	No. (%) of ESBL-producers	No. of isolates	No. (%) of ESBL-producers	No. of isolates	No. (%) of ESBL-producers	No. of isolates (% of total isolates)	No. (%) of ESBL-producers
Escherichia coli	81	41 (50.6)	75	50 (66.7)	102	50 (49.0)	3	2 (66.7)	261 (40.7) [a]	143 (54.8) [b]
Klebsiella spp. [c]	50	30 (60.0)	39	33 (84.6)	52	45 (86.5)	6	4 (66.7)	147 (22.9)	112 (76.2)
Enterobacter spp. [d]	27	17 (63.0)	25	21 (84.0)	9	4 (44.4)	1	1 (100)	62 (9.7)	43 (69.4)
Others[e]	35	14 (40.0)	112	59 (52.7)	14	7 (50.0)	11	8 (72.7)	172 (26.8)	88 (51.2)
Total	193	102 (52.8)	251	163 (64.9)	177	106 (59.8)	21	15 (71.4)	642 (100) [f]	386 (60.1)

ICU, intensive care unit.
[a] Total number of *E. coli* isolated.
[b] Total number of ESBL-producing *E. coli*.
[c] Includes *K. pneumoniae* (n = 136), *K. variicola* (n = 4) and *K. oxytoca* (n = 7).
[d] Includes *E. cloacae* (n = 43), *E. kobei* (n = 4) and *E. asburiae* (n = 15).
[e] Includes *Pseudomonas* spp. (*P. aeruginosa*, n = 39; *P. putida*, n = 2; *P. fulva*, n = 1), *Acinetobacter* spp. (*A. baumannii*, n = 33; *A. baylyi*, n = 1; *A. junii*, n = 2; *A. calcoaceticus*, n = 1; *A. lwoffii*, n = 1; *A. schindleri*, n = 1; *A. radioresistens*, n = 1, *A. haemolyticus*, n = 1; *A. parvus*, n = 1), *Alcaligenes faecalis* (n = 3), *Citrobacter* spp. (*C. freundii*, n = 20; *C. sedlakii*, n = 1; *C. braakii*, n = 2), *Providencia* spp. (*P. stuartii*, n = 10; *P. rettgeri*, n = 9; *P. alcalifaciens*, n = 1), *Proteus* spp. (*P. mirabilis*, n = 8, *P. hauseri*, n = 8; *P. penneri*, n = 1), *Morganella morganii* (n = 11), *Leclercia adecarboxylata* (n = 2), *Serratia rubidaea* (n = 1), *Serratia marcescens* (n = 1), *Comamonas kerstersii* (n = 2), *Stenotrophomonas maltophilia* (n = 1), *Kerstersia gyiorum* (n = 1), *Kluyvera ascorbata* (n = 1), *Salmonella* Newington (n = 1), *Raoultella ornithinolytica* (n = 1), *Aeromonas hydrophilia* (n = 1), *Kosakonia cowanii* (n = 1) and *Pantoea septica* (n = 1).
[f] Total number of Gram-negative bacteria isolated from 1087 clinical samples.

European collection of the ST131/C2 subclone [21] (Supplementary Fig. S1). On the other hand, 4.3% (6/138) were found to be unknown sequence types (Supplementary Table S1).

3.4. Single nucleotide polymorphism (SNP) analysis and population structure

SNPs were called from the shared dynamic core genome (45.5%) for all isolates, which resulted in a 117 343-bp alignment and 66 594 bp remained for phylogenetic inference, after removing recombinant regions. Maximum likelihood tree analysis revealed clusters of clonally related isolates with a similarity index 0 to <10 SNPs difference between isolates from different units of the hospital (Supplementary Table S5). These clusters were found among epidemiologically important sequence types: ST410, ST10, ST44, ST38, ST131, ST405 and two separate clusters of ST648 (Fig. 2).

3.5. Plasmid replicon typing

The most prevalent plasmid replicon types were IncFII(pRSB107) (24.8%; 32/129), IncQ1 (20.2%; 26/129), Col(BS512) (14.0%; 18/129), IncX4 (7.8%; 10/129) and IncI1 (6.2%; 8/129), and in total 15 different replicon types were identified. These plasmid replicons were detected among multiple epidemiologically important sequence types (Supplementary Table S6). In addition, we have previously analysed plasmids from ST410 isolates (n = 24) using optical DNA mapping and comparing with short-read (next generation sequencing), and long-read (Nanopore) sequencing. The analysis demonstrated that the plasmid carrying the $bla_{\text{CTX-M-15}}$ gene was very similar among the ST410 isolates that were also genetically very similar [22].

4. Discussion

We found a high prevalence of $bla_{\text{CTX-M-15}}$-harbouring ESBL-EC from patients with suspected infections in a tertiary hospital in Jimma, Ethiopia. None of the previous studies conducted in Ethiopia focused on the genomic epidemiology of ESBL-EC. In the current study, several clusters of highly related isolates from different wards of the hospital were detected, indicating nosocomial transmission.

The predominant sequence types were epidemiologically important high-risk clones, including ST131-*H*30/C2. This clone has not been reported from Ethiopia before. Another emerging epidemiologically important clone is ST410, and nosocomial transmission was confirmed for ST410 in the present study. On genome comparison with an international collection of ST410 isolates in the EnteroBase database, most of the isolates (n = 15) in the present study clustered in separate groups, but some of the isolates (n = 5) were clustered with international collections of ST410 isolates (Supplementary Fig. S2).

The genotypes of ESBL-EC in Ethiopia were unknown until a study in 2017 demonstrated that the $bla_{\text{CTX-M}}$ genotypes are predominant [11]. However, epidemiologic typing, detection of high-risk clones and nosocomial transmission of these strains have not been well studied in low-income countries. The prevalence of ESBLs among *E. coli* (54.8%) in this study was higher than previous reports from East African countries (39% in Kenya [23], 24.2% in Tanzania [24] and 34% in Uganda [25]). Other β-lactamase genes, such as $bla_{\text{OXA-1}}$ and $bla_{\text{TEM-1B}}$, were also detected at a high prevalence. Moreover, two distinct, but related, plasmid-mediated AmpC (pAmpC) β-lactamases ($bla_{\text{CMY-2}}$ and $bla_{\text{CMY-42}}$) were detected together with the $bla_{\text{CTX-M-15}}$ gene among the ST410 isolates. A previous phenotypic study from Ethiopia reported 1.2% of *E. coli* isolates collected from different laboratories in Addis Ababa were pAmpC-producing [26]. Similarly, low prevalences of $bla_{\text{CTX-M-15}}$

Table 2
Sociodemographic and clinical characteristics of patients and rate of all extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria among patients at Jimma Medical Center, Ethiopia

Characteristic	*N* (%) of patients			*P*-value
	ESBL-positive [a]	ESBL-negative	Total	
Age category	0.000			
≥15 years	228 (26.4)	636 (73.6)	864	
≥5 to <15 years	48 (45.3)	58 (54.7)	106	
≥1 to <5 years	83 (78.3)	23 (21.7)	106	
<1 year	10 (90.9)	1 (9.1)	11	
Total	369 (33.9)	718 (66.1)	1087	
Sex				
Male	214 (33.4)	426 (66.6)	640	0.369
Female	155 (34.7)	292 (65.3)	447	
Admission				
Outpatient	66 (23.2)	219 (76.8)	285	0.000
Admitted	303 (37.8)	499 (62.2)	802	
Currently using antibiotic				
Yes	235 (34.4)	449 (65.6)	684	0.381
No	134 (33.3)	269 (66.7)	403	
Type of specimen				
Sputum	50 (18.7)	217 (81.3)	267	0.000
Wound swab	56 (30.9)	125 (69.1)	181	
Stool	163 (90.6)	17 (9.4)	180	
Urine	100 (21.9)	356 (78.1)	456	
CSF	0	3 (100)	3	
Presence of underlying chronic illness				
Yes	271 (38.5)	432 (61.5)	703	0.000
No	98 (25.5)	286 (74.5)	384	
Service unit at which the patient was recruited/admitted				
ICU	8 (19.0)	34 (81.0)	42	0.000
Paediatric	130 (74.3)	45 (25.7)	175	
Medical	125 (24.6)	384 (75.4)	509	
Surgical	106 (29.4)	255 (70.6)	361	
Clinical diagnosis of suspected infection				
UTI	100 (21.9)	356 (78.1)	456	0.000
Pneumonia	45 (18.4)	200 (81.6)	245	
Diarrhoea	164 (90.6)	17 (9.4)	181	
Wound infection	56 (31.3)	123 (68.7)	179	
COPD	4 (15.4)	22 (84.6)	26	
Total	369	718	1087	

CSF, cerebrospinal fluid; ICU, intensive care unit; UTI, urinary tract infection; COPD, chronic obstructive pulmonary disease.
[a] Number of patients with at least one ESBL-producing isolate.

and bla_{CMY-2} genes were detected from single isolates from studies in Tanzania [27] and Mexico [28].

Despite a high prevalence of resistance to aminoglycosides, fluoroquinolones, sulfonamides, phenicols, trimethoprim and macrolides, these antimicrobials are still commonly prescribed at the hospital. Based on a short survey of drug supply at the hospital, the oldest available record revealed that ceftriaxone was the only third-generation cephalosporin extensively used at the hospital over the last 10 years. The high rate of self-medication and over-the-counter drugs [29], the higher cost of treatment and lack adherence to strict prescription policy [30], and lack of efficient surveillance and containment strategies in the country may worsen this problem.

The coexistence of aminoglycoside and fluoroquinolone resistance genes among the ESBL-producers severely compromises the treatment options for infections. Thus, the use of other classes of antimicrobials such as carbapenems, for which these strains showed susceptibility, may be necessary. Conversely, the use of carbapenem antimicrobials in this region, where prescription is not standardised [31] and antimicrobial stewardship is not strictly implemented [32], bears a higher risk for development and spread of carbapenem resistance.

The IncFII(pRSB107) and IncQ1 plasmids, most prevalent among ESBL-EC isolates in the present study, might have mediated the co-transmission of multiple antimicrobial resistance genes within and between different epidemiological clones (Supplementary Table S6). In a review by Caratolli, IncFII plasmids were reported to encode and carry several resistance genes including $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1} and *aac(6′)-Ib*-cr, and are highly conserved low-copy-number plasmids [33]. The second most prevalent plasmid (IncQ1) is a non-conjugative plasmid with high mobility and stability among Enterobacterales [34]. IncQ1 plasmids encoding various resistance genes have been reported in *Salmonella* and *K. pneumoniae* strains [34,35]. However, the role of IncQ1 plasmids in the transmission of $bla_{CTX-M-15}$ has not been previously reported. Further studies are needed to elucidate the impact of IncQ1 plasmids in the spread of $bla_{CTX-M-15}$. Our previous optical DNA mapping study confirmed that the $bla_{CTX-M-15}$ gene was located on a plasmid in the ST410 strain spreading in the hospital [22].

Most of the isolates classified as pathogenic (either EPEC or ExPEC) were collected from different clinical samples, but the EAEC pathotypes were predominantly isolated from diarrhoea stool samples from paediatric patients. These patients were admitted to the paediatric unit for mild to severe malnutrition. Previous studies also reported EAEC from malnourished children with diarrhoea [27,36,37]. Of these studies, the recent and comprehensive surveillance birth cohort study (MAL-ED) reported the impact of EAEC isolated from non-diarrhoeal faecal samples obtained from children with malnutrition and related the incidence to short falls in the growth of children in early life as a cause of malnutrition [38].

MLST analysis demonstrated the presence of epidemiologically successful clones that can cause extraintestinal infections. The

Table 3
Prevalence and distribution of antimicrobial resistance genes identified among extended-spectrum β-lactamase-producing *Escherichia coli* isolated at Jimma Medical Center, Ethiopia ($n = 138$)

Antimicrobial class	Resistance determinant	Genes detected	*n* (%)[a]
Aminoglycosides	Acetylation	*aac(3)-IIa*	2 (1.4)
		aac(3)-IId	2 (1.4)
		aac(6′)-IIc	1 (0.7)
	Aminoglycosides and fluroquinolones	***aac(6′)-Ib-cr***	**86 (62.3)**
	Adenylation	*aadA1*	8 (5.8)
		aadA2	11 (8.0)
		aadA5	75 (54.3)
		aadB	3 (2.2)
	Streptomycin	***strA***	**71 (51.4)**
		strB	55 (39.9)
	Phosphorylation	*aph(3′)-Ia*	1 (0.7)
β-Lactams	CMY	bla_{CMY-2}	17 (12.3)
		bla_{CMY-42}	2 (1.4)
	CTX-M	$bla_{CTX-M-11}$	4 (2.9)
		$bla_{CTX-M-14}$	6 (4.3)
		$bla_{CTX-M-15}$	**122 (88.4)**
		$bla_{CTX-M-27}$	3 (2.2)
		$bla_{CTX-M-55}$	1 (0.7)
	OXA	**bla_{OXA-1}**	**87 (63.0)**
		bla_{OXA-10}	2 (1.4)
	TEM	bla_{TEM-1B}	75 (54.3)
	SHV	bla_{SHV-12}	1 (0.7)
Carbapenems	NDM	bla_{NDM-1}	1 (0.7)
Phenicols	Chloramphenicol acetyltransferase	*catA1*	6 (4.3)
		catB3	**79 (57.2)**
		catB4	2 (1.4)
Trimethoprim	Dihydrofolate reductase	*dfrA1*	13 (9.4)
		dfrA12	10 (7.2)
		dfrA17	**81 (58.7)**
		dfrA27	3 (2.2)
		dfrA5	7 (5.1)
		dfrA7	5 (3.6)
		dfrA8	2 (1.4)
		dfrB4	1 (0.7)
Fosfomycin	Glutathione transferase	*fosA*	1 (0.7)
Macrolides		***mph(A)***	**93 (67.4)**
Fluoroquinolones	Plasmid-mediated quinolone resistance proteins	*qepA*	1 (0.7)
		qnrB1	1 (0.7)
		qnrB4	1 (0.7)
		qnrB6	1 (0.7)
		qnrS1	**7 (5.1)**
Tetracycline	Tetracycline resistance protein	*tet(A)*	47 (34.1)
		tet(B)	**62 (44.9)**
		tet(D)	1 (0.7)
Sulfonamides	Dihydropteroate synthetase	***sul1***	**95 (68.8)**
		sul2	79 (57.2)
		sul3	1 (0.7)
Rifampicin	Rifampin ADP-ribosyl transferase	*arr-2*	1 (0.7)
		arr-3	**3 (2.2)**
Total			138 (100) [b]

[a] Figures in bold represent the highest prevalence in each group of resistance determinants.
[b] Five isolates were excluded from the analysis of molecular characteristics because the genomic material failed the library preparation during sequencing.

prevalent sequence types in this study (ST410, ST131, ST648, ST10, ST167 and ST405) are internationally recognised and epidemiologically important isolates. Also, a review on the global epidemiology of ExPEC lineages revealed that these sequence types are the predominant isolates from a globally recognised pool of ExPEC isolates [39]. On the other hand, ST410 has so far been reported mainly from Europe and the Americas [40]. It was also recently reported from Southeast Asia [2] but has rarely been reported from Africa [41]. This study presents this newly emerging international high-risk *E. coli* clone from Ethiopia.

ST131-*H*30-Rx/C2, ST2172, ST648, ST405 and ST410 were involved in nosocomial spread in the hospital and were isolated from patients with suspected infections. Nosocomial transmission of high-risk clones owing to lack of infection prevention and control strategies as well as the spread of multidrug-resistant strains with resistance to cephalosporins mediated by β-lactamases compromise patient safety [27,28]. To our knowledge, these clones have not been previously reported from Ethiopia. Other epidemiologically important clones such as ST10, ST405, ST167, ST617, ST130 ST44 and ST38 detected at this hospital were also reported previously from other countries [1,2,39].

Moreover, age <5 years, admission to the hospital, diarrhoea and the presence of underlying chronic illness were the risk factors for acquiring ESBL-EC. These risk factors are also the most common factors contributing to severity of disease. Owing to the persistence of such strains in the hospital environment and in humans, together with the limited resources including unavailability of alternative antimicrobials, the identification of high-risk clones at this hospital bears a huge challenge to clinical care and public health. Furthermore, the high prevalence of ESBL-EC and nosoco-

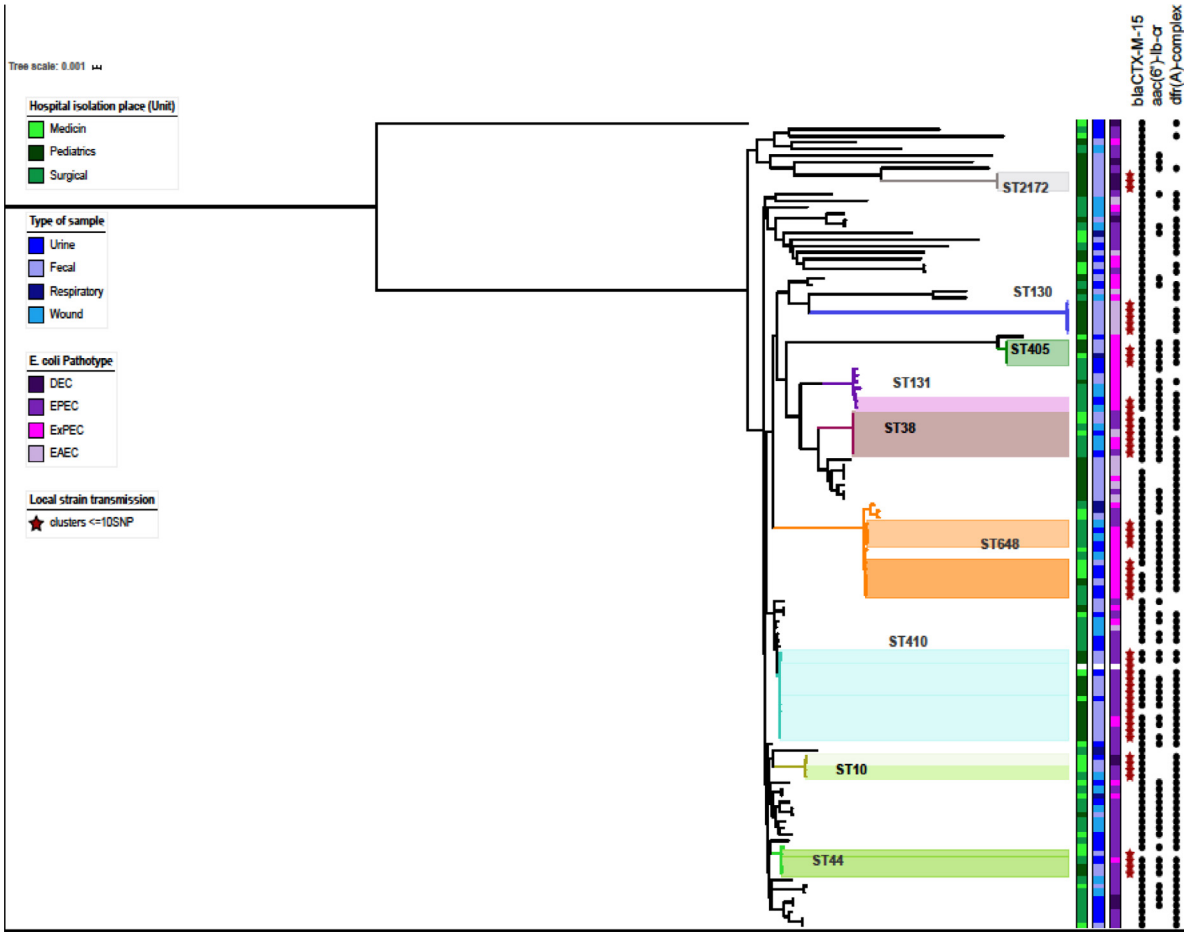

Fig. 2. Maximum likelihood tree inferred with FastTree/2.18 from the dynamic core genome of 129 isolates. Single nucleotide polymorphisms (SNP) clusters of related clones showing nosocomial transmission of ESBL-producing *Escherichia coli* (ESBL-EC) multiple sequence types (STs) isolated from medical, surgical and paediatric wards of the hospital. Antimicrobial resistance genes [$bla_{CTX\text{-}M\text{-}15}$, *aac(6′)-Ib*-cr and *dfrA12*) and different *E. coli* pathotypes as defined by a review conducted on *E. coli* pathotypes by Croxen et al. [20]. Diarrhoeagenic *E. coli* (DEC), enteropathogenic *E. coli* (EPEC), extraintestinal pathogenic *E. coli* (ExPEC) and enteroaggregative *E. coli* (EAEC) were added to the tree.

mial transmission of high-risk clones, in the face of compromised capacity of detection and treatment, may cause an increase in the incidence of severe difficult-to-treat infections.

5. Conclusions

The prevalence of ESBL-producers among *E. coli* isolates isolated at a tertiary hospital in Ethiopia was found to be very high (54.8%). Multidrug-resistant strains and international high-risk clones (ST131, ST167, ST410 and ST648) are emerging. The detection and nosocomial transmission of high-risk clones in Ethiopian hospitals or other low-income countries greatly compromises patient safety and quality of care. These high-risk clones may acquire carbapenem resistance genes and evolve to cause infections that are challenging to treat. Therefore, strengthening microbiology laboratory and surveillance strategies is highly desirable. Engaging in antimicrobial stewardship programmes, infection control, and ensuring strict prescription policy is recommended.

Appendix A. Supplementary data: Supplementary material related to this article can be found, in the online version.

TS: add in link

Declaration of Competing Interest

None declared.

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Ethical approval

This study was conducted after receiving ethical approval from institutional and national level review boards.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.09.017.

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