

# Magnitude and antimicrobial susceptibility patterns of extended-spectrum $\beta$ -lactamase producing *Enterobacteriaceae* recovered from environmental samples at Tikur Anbessa Specialized Hospital, Ethiopia

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## Research article

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## Abstract

## Background

The hospital environment especially the intensive care units (ICUs) contributes to the spread of extended-spectrum  $\beta$ -lactamase (ESBL) producing *Enterobacteriaceae*, which are contributing to increasing morbidity and mortality rates. We aimed to assess the rate of environmental contamination of ESBL producing *Enterobacteriaceae* and their antimicrobial susceptibility patterns.

## Methods

A Cross-sectional study was conducted at Tikur Anbessa Specialized Hospital (TASH) from June to July, 2018. A total of 65 environmental swab were taken from inanimate items. All isolates from the inanimate environment were cultured by using ESBL ChromoSelect Agar and identified using conventional bacteriological methods. All ESBL producing *Enterobacteriaceae* isolates were tested for their antimicrobial susceptibility using the disc diffusion method according to the methods and interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI).

## Results

From a total of 65 environmental swab samples, 16 (24.6%) were ESBL producing *Enterobacteriaceae*. The most predominant ESBL producing isolates were *E. coli* (8/16, 50%) and *Klebsiella* spp (6/16, 37.6%). The Pediatrics and Medical-Surgical ICU exhibited the highest ESBL producing *Enterobacteriaceae*, each with (37.5%, 6/16). Linens and ventilators were associated with high rates of ESBL isolates positivity each with equal 31.3% detection rate. ESBL producing *Enterobacteriaceae* isolates had significantly high resistance level to ampicillin (100%), ceftazidime (93.8%), cefepime (87.5%) and cefotaxime (86.7%). However, low resistance level was recorded against amikacin (18.8%).

## Conclusion

The appearance of ESBL producing *Enterobacteriaceae* among ICUs hospital environment is posing a serious threat for the control of nosocomial infections. The high resistance found from this study shows the need for policies for devising infection control procedures and screening policies for ESBL on a routine basis.

## Background

In Gram-negative bacteria,  $\beta$ -lactamase production remains the most important mechanism of resistance to  $\beta$ -lactam group of antibiotics and has become a global challenge in infection control. One group of these enzymes is called extended-spectrum  $\beta$ -lactamases (ESBLs) [1–3]. ESBLs hydrolyze a wide range of cephalosporins including the oxyimino group of cephalosporins such as ceftriaxone, ceftazidime, cefotaxime and the monobactam drugs such as aztreonam, but do not hydrolyze cephamycins and carbapenems, and their activity is inhibited by clavulanic acid [3, 4].

ESBL producing bacteria have been detected in inanimate surfaces of hospitals which could contribute to their dissemination [5, 6]. The detection of bacterial pathogens including ESBL producing bacteria from inanimate hospital environments is due to an innate ability of bacteria to survive on surfaces for long periods [7]. For instance, *Klebsiella* spp and *Escherichia coli*, which are the predominant ESBL producing *Enterobacteriaceae*, have been reported to survive up to 30 and 16 months, respectively, on inanimate dry surfaces [5].

In Ethiopia the rate of ESBLs on clinical isolates has been increased from time to time [8–10]. The increased rate of ESBL-producing bacteria in Ethiopia may be due to the cross transmission of strains from patient to patient or from the inanimate hospital environment to the patients and from health care workers to patients and vice versa [6]. Moreover, the horizontal transmission of resistance genes through genetic mobile elements has also important role in adaption of bacteria for various conditions in the hospital environments [11, 12]. The contaminated hospital environment in the transmission of ESBL producing *Enterobacteriaceae* is widely considered the principal cause of hospital acquired infections (HAIs) [13].

Despite the recent worldwide spread of ESBL in *K. pneumoniae* and *E. coli* isolates from HAIs, their dissemination has been little studied especially on inanimate hospital environments in Ethiopia. This study was therefore aimed at determining the rate of environmental contamination of ESBL producing *Enterobacteriaceae* and their antimicrobial susceptibility patterns will have a valuable input to control the emerging problem in this direction.

## Methods

### Study design and Environmental Sampling

This cross-sectional study included 65 environmental swabs at Tikur Anbessa Specialized Hospital from June to July, 2018. The samples were collected from four sites including the intensive care units of Surgical, Pediatric, Medical and Medical-Surgical wards of the hospital. The samples were collected on a single occasion, using pre-moistened sterile swabs from environmental surfaces and medical equipment including beds, monitors, linens, ventilators, suction machine, Lobby (furniture), work station and sinks in accordance to suggestions from previous studies [14, 15].

### Culture and Bacterial isolates

The swab samples were enriched overnight at 37 °C in brain heart infusion broth (BHI) (Merck, Germany) [16]. A loop full of bacterial growth was transferred to ESBL ChromoSelect Agar (SIGMA-ALDRICH) and incubated for 24 h at 37°C to assess the ESBL production. Bacterial identification was achieved using specific colony color on ESBL ChromoSelect agar and Gram staining combined with panel of biochemical tests including Triple Sugar Iron agar (TSI), urea, citrate, Sulfide Indole Motility (SIM) medium, Lysine Iron Agar (LIA), mannitol, malonate and oxidase [17].

### Antimicrobial susceptibility testing

Antimicrobial susceptibilities of ESBL producing *Enterobacteriaceae* isolates were determined using disc diffusion method on Mueller-Hinton agar (MHA) (Oxoid, UK) based on Clinical and Laboratory Standards Institute (CLSI) performance standard methods and interpretive criteria [18]. Isolates were tested for susceptibility to the following 13 antibiotics (in µg/disk): ampicillin (10), amoxicillin/ clavulanic acid (20/10), ceftriaxone (30), ceftazidime (30), cefotaxime (30), cefepime (30), aztreonam (30), meropenem (10), gentamicin (10), amikacin (30), ciprofloxacin (5), trimethoprim/ sulfamethoxazole (1.25/23.75) and chloramphenicol (30). Data analysis was performed using SPSS version 25 software program.

### Phenotypic confirmation of ESBL

All the identified isolates were phenotypically confirmed for ESBL production using Combination Disk (CD) method on Mueller-Hinton Agar (MHA) (Oxoid, UK). Briefly, a cefotaxime disc (30 µg) was placed 20 mm away from a disc containing cefotaxime-clavulanic acid (30/10 µg). When the inhibition zone between at least one of the combination discs and its

corresponding single antibiotic disc differed by  $\geq 5$  mm, the strain was identified as an ESBL producer according CLSI guideline [18]. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the control strains.

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [19].

## Ethics approval and consent to participate

The study protocol was reviewed and approved by Department of Microbiology, Immunology and Parasitology Research Ethics Review Committee (DRERC), College of Health Sciences, Addis Ababa University.

## Results

### Culture results and Bacterial profiles

ESBL producing *Enterobacteriaceae* were isolated in 16 of the 65 (24.6%) environmental samples, with *Escherichia coli* (8/16, 50%), *Klebsiella pneumoniae* and *Klebsiella oxytoca* each with (3/16, 18.8%) being the most predominant ESBL producing environmental isolates (Table 1).

Table 1: Frequency of ESBL producing bacteria from inanimate environments at TASH, 2018.

Isolates	Frequency	Percentage (%)
<i>Klebsiella pneumoniae</i>	3	18.8
<i>Escherichia coli</i>	8	50
<i>Enterobacter</i> spp	2	12.5
<i>Klebsiella oxytoca</i>	3	18.8
Total	16	100

### Distribution of ESBL producing *Enterobacteriaceae* across different ICUs

The Pediatrics and Medical-Surgical ICU exhibited the highest ESBL producing *Enterobacteriaceae*, each with (37.5%, 6/16). The major pathogens in these ICUs were *E. coli*, (37.5%, 3/8). Surgical and Medical ICUs were contaminated with *Klebsiella pneumoniae* as high as (33.3%, 1/3) (Table 2).

Table 2: Distribution ESBL producing *Enterobacteriaceae* across different ICUs at TASH, 2018

Isolates	Sites of environmental samples in the ICUs			
	Surgical	Pediatrics	Medical	Medical-Surgical
	N=15 n (%)	N=17 n (%)	N=18 n (%)	N=15 n (%)
<i>Klebsiella pneumoniae</i>	1(6.7)	1(5.9)	1(5.6)	
<i>E. coli</i>	1(6.7)	3(17.6)	1(5.6)	3(20)
<i>Enterobacter</i> spp				2(13.3)
<i>Klebsiella oxytoca</i>		2(11.8)		1(6.7)
Total (n=16)	2(12.5)	6(37.5)	2(12.5)	6(37.5)

## Distribution of ESBL producing *Enterobacteriaceae* over different surfaces

The highest number of ESBL producing bacteria were obtained from linens and ventilators each, with 5(31.3%) followed by monitor and bed, each with 2(12.5%) (Table 3).

Table 3: Distribution of ESBL producing *Enterobacteriaceae* over different surfaces at TASH, 2018

Sites	Isolates n (%)				Total =16
	<i>Klebsiella pneumoniae</i>	<i>E. coli</i>	<i>Enterobacter spp</i>	<i>Klebsiella oxytoca</i>	
Monitor		1	1		2(12.5)
Sink			1		1(6.3)
Bed		2			2(12.5)
Ventilator	2	2		1	5(31.3)
Linens	1	2		2	5(31.3)
lobby (furniture)		1			1(6.3)
Total =16	3(18.8)	8(50)	2(12.5)	3(18.8)	16(100)

## Antimicrobial susceptibility pattern of ESBL

The Extended spectrum  $\beta$  lactamase (ESBL) producing *Enterobacteriaceae* isolates obtained from this study were found to show significantly high resistance level to ampicillin (100%), ceftazidime (93.8%), cefepime (87.5%), azetronome (81.3%), and cefotaxime (86.7%) and amoxicillin with clavulanic acid (81.3%). Low resistance level was recorded for amikacin (18.8%) followed by gentamicin and meropenem each with (37.5%). Among the most common isolated ESBL, *E. coli* showed a higher rate of resistance to ampicillin (100%), ceftazidime (87.5%), ceftriaxone (87.5%) and cefotaxime (87.5%). Furthermore, non-beta lactam antimicrobials such as ciprofloxacin and sulfamethoxazole-trimethoprim resistance was observed in 37.5% and 75% of *E. coli* isolates, respectively. *K. pneumoniae* revealed high resistance (100%) to ampicillin, ceftriaxone, ceftazidime, cefepime, amoxicillin with clavulanic acid and sulfamethoxazole-trimethoprim (Table 4).

Table 4: Antimicrobial susceptibility pattern of ESBL producing bacteria from inanimate environments at TASH, 2018.

Isolates	Ptn	Antimicrobial agent's n (%)												
		AMP	AZM	CTX	CRO	CTZ	FEP	AMC	CHL	MRP	AK	GEN	CIP	SXT
<i>K. pneumoniae</i>	R	3(100)	2(66.7)	2(66.7)	3(100)	3(100)	3(100)	3(100)	1(33.3)	2(66.7)	1(33.3)	1(33.3)	1(33.3)	3(100)
	S	0(0)	1(33.3)	1(33.3)	0(0)	0(0)	0(0)	0(0)	2(66.7)	1(33.3)	2(66.7)	2(66.7)	2(66.7)	0(0)
<i>E. coli</i>	R	8(100)	6(75)	7(87.5)	7(87.5)	7(87.5)	6(75)	5(62.5)	3(37.5)	2(25)	1(12.5)	3(37.5)	3(37.5)	6(75)
	S	0(0)	2(25)	1(12.5)	1(12.5)	1(12.5)	2(25)	3(37.5)	5(62.5)	6(75)	7(87.5)	5(62.5)	5(62.5)	2(25)
<i>Enterobacter spp</i>	R	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)	0(0)	1(50)	2(100)	2(100)
	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	2(100)	1(50)	0(0)	0(0)
<i>K. oxytoca</i>	R	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	2(66.7)	1(33.3)	1(33.3)	1(33.3)	2(66.7)	2(66.7)
	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33.3)	2(66.7)	2(66.7)	2(66.7)	1(33.3)	1(33.3)
<b>Total</b>	R	8(100)	13(81.3)	14(86.7)	15(93.8)	15(93.8)	14(87.5)	15(81.3)	8(50)	6(37.5)	3(18.8)	6(37.5)	8(50)	13(81.3)
	S	0(0)	3(18.7)	2(13.3)	1(6.3)	1(6.3)	2(12.5)	1(18.7)	8(50)	10(62.5)	13(81.3)	10(62.5)	8(50)	3(18.8)

n: number of tested strains; %: percentage; R: resistance; S: Sensitive; Ptn: Pattern; AMP: ampicillin; AZT: Aztreonam; CTX : cefotaxime; CRO: ceftriaxone; CTZ: ceftazidime; FEP: Cefepime; AMC: Amoxicillin and clavulanic acid ; CHL: Chloramphenicol; MRP: Meropenem; AK: Amikacin; GEN: Gentamicin; CIP: ciprofloxacin; SXT: Sulfamethoxazole + trimethoprim

## Distribution of MDR phenotypes

Of the total 16 ESBL producing *Enterobacteriaceae* isolates, 15(93.8%) were resistant to at least 3 antibiotics and twelve multi-drug resistance phenotypes were detected. The predominant MDR phenotype was observed in *E. coli* (8/8, 100%), *Klebsiella pneumonia* (3/3, 100%) and *Enterobacter spp* (2/2, 100%). The most common MDR combinations found among *E. coli* isolates was against 7 classes of antibiotics (Table 5).

Table 5: Multidrug-resistance profiles of ESBL producing *Enterobacteriaceae* at TASH, 2018.

MDR phenotype	Frequency, n (%)
<b><i>E. coli</i></b>	
AMP, CTX, AMC, MRP, AK, CIP, SXT	2(25)
AMP, CTX, SXT	2(25)
AMP, CTX, AMC	2(25)
AMP, CTX, AMC, AK, CIP, CHL, SXT	1(12.5)
AMP, CTX, AMC, SXT	1(12.5)
<b>Total</b>	<b>8/8(100)</b>
<b><i>Klebsiella pneumonia</i></b>	
AMP, CTX, AMC, GEN, SXT	1(33.3)
AMP, CTX, AMC, SXT	1(33.3)
AMP, CTX, AMC, MRP, AK, CIP, SXT	1(33.3)
<b>Total</b>	<b>3/3(100)</b>
<b><i>Klebsiella oxytoca</i></b>	
AMP, CTX, AMC, MRP, AK, CIP, STX	1(33.3)
AMP, CTX, AMC, AK, CIP, CHL, SXT	1(33.3)
<b>Total</b>	<b>2/3(66.7)</b>
<b><i>Enterobacter spp</i></b>	
AMP, CTX, AMC, MRP, AK, CIP, SXT	1(50)
AMP, CTX, AMC, GEN, CIP, SXT	1(50)
<b>Total</b>	<b>2(100)</b>

AMP: ampicillin; CTX : cefotaxime; AMC: Amoxicillin and clavulanic acid ; CHL: Chloramphenicol; MRP: Meropenem; AK: Amikacin; CIP: ciprofloxacin; SXT: Sulfamethoxazole + trimethoprim, MDR: Multi Drug Resistant

## Discussion

In our study, ESBL producing *Enterobacteriaceae* were isolated in 16 of the 65 (24.6%) environmental samples. In published reports, the percentage of inanimate hospital environments contaminated with ESBL are significantly varied ranging from 3% to 33% of surfaces in patients' rooms on regular hospitals wards, as reported from Gonder in Ethiopia (14.8%) [6], Tunisia (4%) [11], Israel (9%) [20], Tunisia (4%) [5], UK (3.1%) [12] and Pakistan (33%) [2]. These discrepancies could possibly be due to differences in patient colonization load, hospital's cleaning/disinfection protocols, study design including timing of sample collection and laboratory method used [5, 11, 20, 21].

We found a high proportion of ESBL producers among *E. coli* and *K. pneumoniae* isolates which has similarly been reported from studies in Gondar, Ethiopia [6], Zimbabwe [22], Gaza in Palestine [23] and Algeria [24]. Given the fact that *Klebsiella* spp and *Escherichia coli* have capacity to survive up to 30 and 16 months, respectively, on inanimate dry surfaces [5]. Moreover, isolation of such *Enterobacteriaceae* is highly indicative of fecal contamination and poor hand washing practices amongst health workers and patients [25].

The most prevalent ESBL producer detected in the current study was *E. coli* (50%) as compared to *K. pneumoniae* (18.8%) which has similarly been reported from studies in Gaza, Palestine (*E. coli* 40% vs *K. pneumoniae* 20%) [23]. In contrast to our result, different findings from most reports in other parts of the world ESBL production was more predominantly found among *Klebsiella* spp isolates: Zimbabwe (*Klebsiella* spp 60% vs *E. coli* 40%) [22], Algeria (*K. pneumoniae* 48.5% vs *E. coli* 22.8%) [24], Gondar, Ethiopia (*K. pneumoniae* 42.1% vs *E. coli* 35.1%) [6], Sudan (*K. pneumoniae* 37% vs *E. coli* (0%) [26] and on clinical isolates from another study in Ethiopia (*K. pneumoniae* 72.7% vs *E. coli* 22.7%) [9].

The Pediatrics and Medical-Surgical ICU exhibited the highest ESBL producing *Enterobacteriaceae*, each with (37.5%, 6/16). This may lead to cross-infections between patients in the same ICU because patients present in these ICUs are likely to have reduced immune system due to illness, surgical and mechanical manipulation, and/or the use of immune-suppressors and other therapeutic drugs, all of which increasing patients susceptibility to infections [27, 28].

Bed linens, ventilators, beds and sink were inanimate surfaces observed to be associated with variable degree of ESBL producing *Enterobacteriaceae* positivity, with 31.3%, 12.5% and 6.5%, respectively. The identification of ESBL producing bacteria on linens, sink, bed and other environmental surface is consistent with reports from the literatures [5, 6, 12, 20]. It is generally assumed that ESBL producing Gram-negative bacteria require moist or damp sites for enhanced longevity [12, 24]. Moreover, bed linens are considered to be high patient-contact surfaces and therefore the detected pathogens might have been shed by the infected/colonized patients occupying the particular beds.

In regards to level of antimicrobial resistance among our ESBL producing isolates, significantly high resistance level was recorded to penicillin groups such as ampicillin (100%) and cephalosporin groups such as cefuroxime (96%), ceftazidime (92%), cefepime (86%), azetronome (80%) and cefotaxime (72%). These results were mostly comparable with results from other studies conducted in Ethiopia (cefpirome, cefpodoxime, ceftazidime, ceftriaxone and amoxicillin with clavulanic acid each with 100% resistance level) [6] and Algeria where ampicillin resistance level was reported at 98.1% [24]. Probably lack of antibiotic resistance screening and confirmatory testing prior to using these drugs could be responsible for accumulation of such high level of resistance among ESBL producing bacteria in the hospital environments.

High resistance rate was also recorded from ESBL-producing isolates for non-beta-lactam antimicrobials such as, cotrimoxazole (81.3%), ciprofloxacin and chloramphenicol each with (50%). This was in close agreement with studies done in Ethiopia (ciprofloxacin 56%) [6], from another study in Addis Ababa from clinical isolates (cotrimoxazole 77%, ciprofloxacin 46.3%) [8]; Tikur Anbessa Specialized Hospital, from clinical isolates, cotrimoxazole (83.6%) and Chloramphenicol (61.8%) [9]. The most active drugs for ESBL-producing isolates were amikacin (81.2%). Low resistance rate of amikacin in our study could be explained by the dearth in usage of amikacin in our study setting.

Reports from other studies showed that multidrug resistance (MDR) to the commonly prescribed antimicrobial agents are more common among ESBL producers found from inanimate hospital surfaces [29, 30]. Hence, unwise use of antibiotics

can inadvertently favor emergence of multidrug resistant bacterial strains [11, 28]. The finding from the current study showed that (15/16, 93.8%) of the ESBL producing bacterial isolates were resistant to at least 3 antibiotics, showing how rampant MDR *Enterobacteriaceae* are in the hospital environments. This finding is higher than reports from Northwest Ethiopia (75%) [31], Zimbabwe (75%) [22] and Iran (79.4%) [32].

## Conclusions

In this study the appearance of ESBL producing *Enterobacteriaceae* among ICUs hospital environment in the study site poses a serious threat for the control of nosocomial infections. Occurrence of multidrug resistance (MDR) to the commonly prescribed antimicrobial agents is found to be more common among ESBL producers, showing the need for policies to prevent misuse and overuse of antimicrobial agents. Moreover, it also indicates the need for infection control procedures and screening policies for ESBL on a routine basis in the hospital environment.

## Abbreviations

TASH: Tikur Anbessa specialized Hospital; ICUs: Intensive care units; ESBL: Extended-spectrum  $\beta$ -lactamase; CD: combination disk; %: Percent; °C: Degree Celsius; ATCC: American Type Culture Collection; CLSI: Clinical Laboratory Standards Institute; HAIs: Hospital Acquired Infections; MDR: Multi Drug Resistant; DRERC: Department of Microbiology, Immunology and Parasitology Research Ethics Review Committee; TSI: Triple Sugar Iron agar, SIM: Sulfide Indole Motility medium, LIA: Lysine Iron Agar, MHA: Mueller-Hinton Agar

## Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Department of Microbiology, Immunology and Parasitology Research Ethics Review Committee (DRERC), College of Health Sciences, Addis Ababa University (Ref. no. DRERC/17/18/02-G). Prior to sample collection, written approval was obtained from administrative unit of Tikur Anbessa Specialized Hospital. However, consent was not obtained because it was inanimate environmental samples.

### Consent for publication

Not applicable.

### Availability of data and materials

The dataset supporting the findings of this article is available from the corresponding author upon request.

### Competing interests

The authors declare that they have no competing interests.

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### Author Contributions

All authors have read and approved the manuscript.

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