

Antimicrobial Susceptibility Profile of Extended Spectrum Beta- lactamases Producing *Enterobacteriaceae* isolated from clinical samples refereed to the National Bacteriology and Mycology Reference Laboratory, Ethiopia

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Abstract

Background: Extended-spectrum beta-lactamase producing *Enterobacteriaceae* are very commonly reported all over the world. They are known to cause a huge challenge in the treatment and control of bacterial infectious diseases in many health care facilities. These genes not only confer resistance to oximino-cephalosporins and aztreonam but also, they make bacterial pathogens multidrug-resistant to other commonly available antimicrobial agents.

Objective: To determine the prevalence and antimicrobial susceptibility profile of Extended-spectrum beta-lactamase producing *Enterobacteriaceae* isolates recovered from clinical samples referred to the national clinical bacteriology and mycology reference laboratory of the Ethiopian Public Health Institute.

Methods: A cross-sectional study was conducted on 371 *Enterobacteriaceae* culture- positive clinical specimens that were referred from August 2018 to July 2019. Bacterial isolation was performed according to the inoculation and incubation conditions of each clinical specimen and identifications of the isolates were performed using standardized biochemical tests for gram-negative bacteria. Antimicrobial susceptibility profiles of the isolated bacteria were determined using the disk diffusion method on Muller Hinton agar and the microbial production was detected using Clinical and Laboratory Standard Institute Screening and confirmation test. A double-disk synergy test was used for confirmation. Descriptive statistics was used to analyze the data.

Results: Out of the 371 clinical specimens 240(64.7%) were positive for: extended-spectrum beta-lactamase production and the most prevalent species were *Klebsiella spp* 131(54.6%) followed by *E. coli* 79 (32.9%). Of the positive *Klebsiella spp*, 95 (72.5%) were obtained from blood specimens. Among the 79 *E. coli* isolates, 51 (64.6%) of them were isolated from urine. All the identified positive isolates were resistant to ampicillin and first to third generation cephalosporins. Less resistance rates were documented for carbapenems and amikacin.

Conclusion: The prevalence of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in this study was found to be alarmingly very high with all of them being multidrug resistant to most of the clinically available antimicrobial agents. Instituting a regular surveillance system that can catch such multidrug resistant pathogens and the implementation of antimicrobial stewardship and infection prevention and control measures in health care facilities is highly warranted.

Key words: Enterobacteriaceae, antimicrobial resistance, extended spectrum beta-lactamases, Ethiopia

Introduction

Pathogenic organisms resistant to antimicrobial agents have become a worldwide public health threat because of their great impact on morbidity and mortality. These pathogens are also known to have a significant implication on factors such as length of hospital stay, treatment and diagnosis costs. The most worrisome infections are those that are caused by multidrug resistant organisms showing resistance to various antimicrobial agents commonly used in clinical practices (Li and Thomas 2018).

Enterobacteriaceae producing extended spectrum beta-lactamases (ESBL) are known to have multidrug resistance traits which put challenge on our ability to treat patients infected by such pathogens. These bacterial pathogens are the main agents of most

hospital and community acquired infections throughout the world (Rodríguez-Baño et al. 2018).

ESBL producing organisms are resistant to the action of extended spectrum cephalosporins such as third and fourth generation cephalosporins and monobactams such as aztreonam (Rawat et al. 2010). ESBLs are encoded on plasmids born genes located on integrons and transposons which facilitate the dissemination of these resistance genes within or between different genera of bacteria (Partridge et al. 2018). The genes that encode resistance determinants for other classes of antimicrobial agents are also carried on plasmids encoded ESBL genes. These includes resistant genes for trimethoprim, sulphonamides, tetracyclines, aminoglycosides, chloramphenicol and fluoroquinolones (Rozwandowicz et al. 2018).

ESBLs producing bacteria may also hydrolyze carbapenems drugs including imipenem, meropenem and ertapenem. This is commonly true for ESBL positive organisms that produce *Klebsiella pneumoniae* carbapenemase (KPC) when ESBL production is associated with chromosomal AmpC-type beta-lactamases (Eltai et al. 2018). Therefore, *Enterobacteriaceae* harboring ESBLs are multidrug resistant for multiple antimicrobial classes with very limited treatment options available which significantly impact patient outcomes (Lewis et al. 2007).

In Ethiopia, several studies have been conducted that revealed alarming level of bacterial diversity in various clinical specimens with the majority of those isolates being multidrug resistant (MDR) (Muhie 2019; Mulu et al. 2017). However, the studies that show the impact of ESBL production on the susceptibility patterns of commonly used antimicrobial agents in clinical practice are limited. Therefore, this study was conducted to investigate the occurrence and antimicrobial resistance profile of ESBL producing *Enterobacteriaceae* isolated from clinical specimens referred to the National Clinical Bacteriology and Mycology reference laboratory of the Ethiopian Public Health Institute.

Materials and Methods

Study area: A cross-sectional study was done on culture positive routine clinical specimens for *Enterobacteriaceae* from August 2018 to July 2019 at Clinical Bacteriology and Mycology National Reference Laboratory (NRL), Ethiopian Public Health Institute. The specimens were obtained as part of the routine diagnostic service at the reference laboratory. NRL is a biosafety level two laboratory that serve as a reference laboratory for hospitals. The laboratory is accredited for identification and antimicrobial susceptibility tests on bacteria isolated from various specimens by Ethiopian National Accreditation Office (ENAO). Ethical clearance for this study was obtained from the Ethiopian Public Health institute scientific and ethical review committee.

Bacterial culture and identification: *Enterobacteriaceae* included in this study were obtained from five clinical specimen types namely, blood, body fluids, pus, sputum and urine. They were routinely cultured according to the standardized laboratory protocol for each specimen. A total of 371 clinical specimens were used for laboratory analysis.

Specimens were collected as part of the routine referral system for microbiology diagnostic test from different health facilities in Addis Ababa. The identification of *Enterobacteriaceae* from positive specimens was performed using colony morphology

on culture plates and conventional biochemical techniques according to the laboratory guideline used for the identification of Gram negative bacterial pathogens. Briefly, specimens were inoculated on culture media (macconky, blood and chocolate agar) depending on the specimen types and suspected colonies were subject to a battery of bio-chemical tests including motility, TSI, urea, citrate and LIA.

Antimicrobial Susceptibility tests: Antimicrobial susceptibility test was performed using standardized Kirby Bauer disk diffusion method on Mueller Hinton agar (Oxoid LTD, Basingstoke, Hampshire, England) as recommended by the Clinical and Laboratory Standard Institute (CLSI 2018). Antibiotics agents that were tested in this study include; ampicillin (10 µg), piperacillin (100 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), piperacillin-tazobactam (100/10 µg), amoxicillin-clavulanic acid (20/10 µg), doripenem (10 µg), ertapenem (10 µg), imipenem (10 µg), meropenem (10 µg), cefazolin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg) and Sulphamethoxazole-Trimethoprim (23.75/1.25 µg). Antibiotics susceptibility results were interpreted according to the CLSI zone size interpretive standards. *E. coli* ATCC 25922 was used as control organism for antibiotic susceptibility testing. Multidrug resistant was defined according to the guideline combined by the European Center for Disease prevention and Control (ECDC) and the Center for Disease Control and Prevention (CDC). Accordingly, bacterial isolates that were non susceptible to at least one antimicrobial agent in three different classes of drugs were considered as MDR (Magiorakos et al. 2012).

Phenotypic Detection of ESBL producing Enterobacteriaceae: All *Enterobacteriaceae* resistant to third generation cephalosporins (cefotaxim, and ceftazidim) and fourth generation cephalosporin (cefepim) were classified as ESBL producer and further detected for ESBL production according to the Clinical and Laboratory Standards Institute recommended double disk synergy test (CLSI 2018). Briefly, ESBL producers were detected by using a disc containing cefotaxime (CTX, 30 µg) or ceftazidime (CAZ, 30 µg) in combination with and without clavulanate (CLA, 10 µg) (Oxoid; UK). The increase in zone size diameter by a $5 \geq$ mm for CTX/CLA and CAZ/CLA when compared with those CTX and CAZ alone was determined as the presence of ESBL. *E. coli* ATCC 25922 ESBL negative and *K. pneumoniae* ESBL positive were used as reference strains.

Data analysis: The data were entered and cleaned using Microsoft excels and imported to SPSS version 20.0. The frequency of total culture positive, ESBL producers and corresponding clinical specimen were calculated. Cross-tabulation was used to present the different relation between data categories. Proportions and the actual number of *Enterobacteriaceae* and the susceptibility patterns of the isolates were used to describe frequency of categorical variables. The data were presented in table and graphs.

Results

Bacterial Strains: A total of 371 clinical specimens that were culture positive for *Enterobacteriaceae* were considered for this study. Among these, 240 (64.7%) of the bacterial isolates had a positive screening tests for ESBL production. The most prevalent species was *Klebsiella spp.* 131 (54. 5%) followed by *E. coli* 79 (32.9%), *Enterobacter spp.* 16 (6.7%), *Proteus spp.* 11 (4.6%) and *Citrobacter spp.* 3 (1.3%) (Table 1).

Table 1: Prop of ESBL positive *Enterobacteriaceae* according to sex and age of study participants

Variables			Total bacterial isolates (n = 240)				
			<i>Citrobacter spp.</i> N = 3	<i>E. coli</i> N = 79	<i>Enterobacter spp.</i> N = 16	<i>Klebsiella sp</i> N = 131	<i>Proteus spp.</i> N = 11
			n (%)	n (%)	n (%)	n (%)	n (%)
Sex	Female	100(41.7)	2 (75.0)	36 (45.6)	4 (25.0)	57 (43.5)	1 (9.1)
	Male	140 (58.3)	1 (25.0)	43 (54.4)	12 (75.0)	74 (56.5)	10 (90.9)
Age group	≤ 1 year	84 (35.0)	0 (0.0)	5 (6.3)	3 (18.8)	76 (58.0)	0 (0.0)
	> 1–20	25 (10.4)	0 (0.0)	12 (15.2)	0 (0.0)	11 (8.4)	2 (18.1)
	21–30	32 (13.3)	1 (33.3)	10(12.7)	4 (25.0)	10 (7.6)	3 (27.3)
	31–40	27 (11.3)	1 (33.3)	11 (13.9)	4 (21.4)	12 (9.2)	3 (27.3)
	41–50	23 (9.6)	1 (33.3)	14(17.7)	2 (12.5)	6 (4.6)	0 (0.0)
	51–60	14 (5.8)	0 (0.0)	8 (10.1)	1 (6.3)	5 (3.8)	0 (0.0)
	> 60	35 (14.4)	0 (0.0)	19 (24.1)	2(12.5)	11 (8.4)	3 (27.3)

With respect to patients' demographic variables among positive culture results, the frequency of males infected with ESBL producing isolates were more than females with 140 (58.3%) and 100 (41.7%), respectively. The mean age of the study participants was 27.1 SD±25.7 years. The majority of infected patients with ESBL positive *Enterobacteriaceae* were young children ≤ 1 year of age (35.0%) followed by

the elderly > 60 years (14.4%) (Table 1).ESBL producing *Enterobacteriaceae* strains were mainly found in blood 111 (46.3%), urine 74 (30.8) and pus 37 (15.4%). *Klebsiella spp.* was the most predominant bacterial strains isolated from blood samples while *E. coli* was predominantly recovered from patients with urinary tract infections (Figure 1).

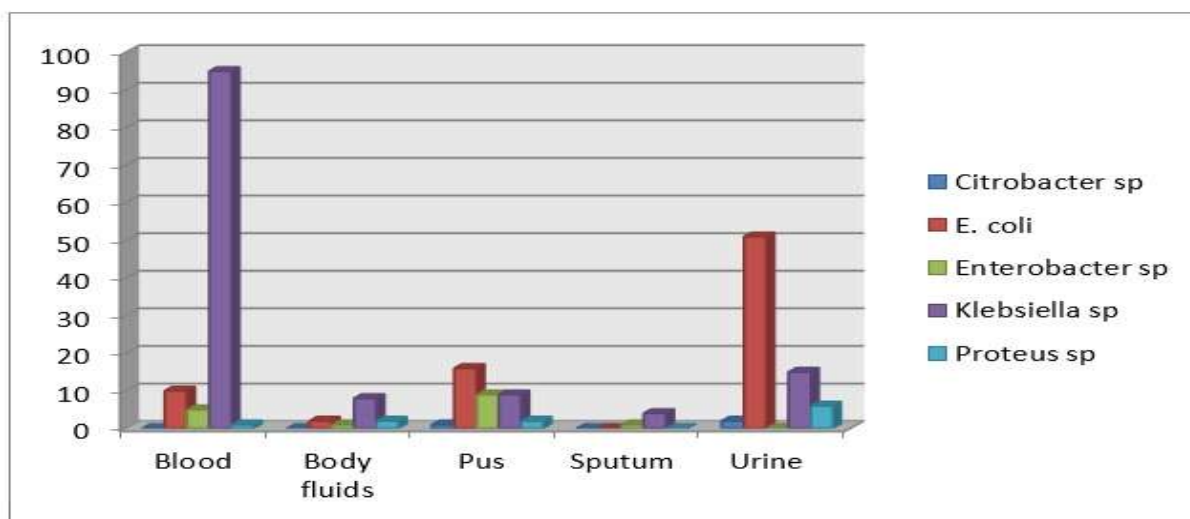


Figure 1: The proportion of ESBL positive *Enterobacteriaceae* species isolated from different clinical specimens

Antimicrobial resistance and ESBL production in *Enterobacteriaceae*: The antimicrobial susceptibility results were analyzed for 240 ESBL positive and 131 ESBL negative isolates. Accordingly, 100% of the

ESBL positive isolates were resistant to ampicillin and all generations (first to fourth) of cephalosporins. The non-ESBL isolates have also shown very high resistance rate to ampicillin (82.4%).

The high percentage of ESBL producing isolates also showed resistance to other antimicrobial agents particularly for piperacillin (95.4%), sulphamethoxazole-trimethoprim (92.1%), and tetracycline (88.3%). The non-ESBL isolates have also shown significant rate of resistance to these antimicrobial agents with 66.4% for piperacillin, 64.9% for sulphamethoxazole-trimethoprim and tetracycline, and 53.4% for amoxicillin-clavulanic acid. Compared to ESBL negative isolates, ESBL positive isolates have shown higher resistance for ciprofloxacin (Table 2).

Table 2: The comparison of the rate of resistance to different antimicrobial agents between ESBL positive and ESBL negative Enterobacteriaceae isolated from clinical specimens

Antimicrobial agents	Number of Enterobacteriaceae (n = 371)		P-value
	ESBL positive (n = 240)	ESBL negative (n = 131)	
	N (%)	N (%)	
AMP	240 (100)	108 (82.4)	< 0.001
PIP	229 (95.4)	87 (66.4)	
AMC	213 (88.8)	70 (53.4)	
TZP	147 (61.3)	44 (33.6)	
CZO	240 (100)	45 (34.4)	
CXM	240 (100)	45 (34.4)	
CRO	240 (100)	0 (0.0)	
CAZ	240 (100)	0 (0.0)	
CTX	240 (100)	0 (0.0)	
CEF	240 (100)	0 (0.0)	
IMP	28 (11.7)	2 (1.5)	
MEM	23 (9.8)	2 (1.5)	
CIP	180 (75.0)	62 (47.3)	
NOR	222 (92.5)	66 (50.4)	
TTC	212 (88.3)	85 (64.9)	
NIT	78 (32.5)	25 (19.1)	
GEN	140 (58.3)	11 (8.4)	
TOB	111 (46.3)	23 (17.6)	
AMK	19 (7.9)	2 (1.5)	
SXT	221 (92.1)	85 (64.9)	
MDR	240 (100)	64 (48.9)	

AMP: ampicillin; PIP: piperacillin; AMC: amoxicillin-clavulanate; TZP: piperacillin-tazobactam; CZO: cefazolin; CXM: cefuroxime; CRO: ceftriaxone; CAZ: ceftazidime; CTX: cefotaxime; CEF: cefepime; MER: meropenem; CIP: ciprofloxacin; NOR: norfloxacin; TTC: tetracycline; NIT: Nitrofurantoin; GEN: gentamicin; TOB: tobramycin; AMK: amikacin; SXT: trimethoprim-sulfamethoxazole

Norfloxacin and nitrofurantoin were only tested for urine samples isolates and a very high percentage (92.5%) of ESBL positive strains showed reduced susceptibility to norfloxacin while only 50.4% of the non-ESBL producing isolates were resistant to this agent. The isolates showed relatively lower resistance to nitrofurantoin. However, the resistance rate of the

isolates to this antibiotic is still higher in ESBL positive organisms.

The susceptibility rate to carbapenem antibiotic agents such as imipenem and meropenem and amikacin from the class of aminoglycoside were more than 80%. However, in comparison with non-ESBL positive isolates, ESBL positive isolates were highly resistant to these classes of antibiotics. In general, ESBL positive *Enterobacteriaceae* were highly resistant to all classes of antimicrobial agents tested as compare with non-ESBL isolates in which the association was statistically significant ($P < 0.001$). Moreover, 100% of ESBL positive *Enterobacteriaceae* analyzed in this particular study were MDR (Table 2).

Discussion

Phenotypic screening of ESBL producing *Enterobacteriaceae* is essential for understanding the development of resistance mechanisms in these pathogens and for proper management of patients in clinical settings as well as for epidemiology purposes (Shaikh et al. 2015). In Ethiopia, various fragmented studies have been conducted regarding ESBL producing *Enterobacteriaceae* in different parts of the country (Muhie 2019; Mulu et al. 2017). The present study was conducted at the National Clinical Bacteriology and Mycology reference laboratory. The data obtained from this study may indicate the level of ESBL burden and its implication on antimicrobial resistance management in the country.

In the current finding, ESBL production was detected in different genera of *Enterobacteriaceae* with overall prevalence of 64.7% in the strains tested with *Klebsiella sp* and *E. coli* being among the majority. This finding is higher than previous reports of studies that were conducted in Ethiopia: Jimma (51%) (Gashaw et al. 2018), Addis Ababa (57.7%) (Teklu et al. 2019) and elsewhere: Algeria (47.6%) (Nedjai et al. 2013), and comparable with the report from Nigeria (65%) (Olowo-okere et al. 2018). The current finding, however, was lower than a report from a tertiary care hospital in Riyadh capital Saudi Arabia (72%) (Marie et al. 2013). These may indicate that the distribution of ESBL producing organisms vary from region to region and may be higher in certain geographic areas than others (Winokur et al. 2001). Regarding the clinical samples analyzed in this study, higher rate of ESBL producers were identified from blood samples with the most commonly recovered isolates being *Klebsiella spp.* followed by *E. coli* and *Enterobacter spp.* similar findings have been reported from Bahirdar Ethiopia (Moges et al. 2019). These bacterial pathogens are the major Gram negative isolates responsible for bloodstream infections.

The incidence of these pathogens was particularly very high among pediatrics population with age group of ≤ 1 year. The finding from the current study was in agreement with another study from Ethiopia (Dagneu et al. 2013) and another country (Hojsak et al. 2012). This suggests that the risk of children acquiring bloodstream infections is greater due to different factors. Such factors include: underdeveloped immune system in the young children, poor skin integrity, frequent visit to health care facility, and the parents' socioeconomic status, poor hygiene practices and high incidence of delivery at home particularly in developing country (Hojsak et al. 2012).

High rate of the ESBL producing *Enterobacteriaceae* were identified from elderly patients greater than 60-year-old group as well. This may be explained by the longer exposure of these individuals to extended spectrum cephalosporins drugs which has been well described as the age of the patients are one of the factors for antimicrobial resistance (Garcia et al. 2017).

Bacteria characterized in the present study showed varied degree of antimicrobial susceptibility with majority of them having high level of resistance to all tested antimicrobial agents. The majority of these bacteria are found to be ESBL producers. In addition to their ability to hydrolyze the activity of common beta-lactams antimicrobial agents such as penicillins, cephalosporins and aztreonam, ESBLs producing pathogens are also known to have resistant traits for other antimicrobial classes and as a result they manifested a multidrug resistance trait (Thenmozhi et al. 2014).

Based on the present finding, ESBL producing *Enterobacteriaceae* showed high resistance rate to amoxicillin-clavulanate, the drug that is proved to inhibit the action of ESBL enzyme. This may be explained by the production of chromosomal beta-lactamases (AmpC) with serine active sites which have the ability to hydrolyze cephalosporins and also making bacterial pathogens resistant to beta-lactamases inhibitors including clavulanate, sulbactam and tazobactam (Silva and Lincopan 2012).

In comparison to amoxicillin-clavulanate, piperacillin-tazobactam combination was relatively active against ESBL producing bacterial pathogens in the present study. This was justified in a review conducted by Drawz and Bonomo in that piperacillin in combination with beta-lactamases inhibitors was resistant to the hydrolysis of some plasmid mediated beta-lactamases as compared with amoxicillin or ampicillin combination with beta-lactamases inhibitors (Drawz and Bonomo 2010).

Fluoroquinolones such as ciprofloxacin and norfloxacin are used for treatment of various bacterial infections and are among the therapeutic options for infections caused by ESBL positive organisms (Dallenne et al. 2010). Fluoroquinolones resistance rate of ESBL producing *Enterobacteriaceae* is described in another study (Chen et al. 2012) which is in agreement with the finding from the present study that 75% and 92.5% of ESBL positive isolates were resistant to ciprofloxacin and norfloxacin, respectively as compared to 47.3% and 50.4% resistance rates for non ESBL producers to the agents, respectively. This is due to the fact that plasmids encoded ESBL genes also carry plasmid mediated quinolone resistance genes. As resistance plasmids with ESBLs encoding genes are transferred among different species of *Enterobacteriaceae* by conjugation and this helps for the dissemination of plasmid mediated quinolones resistance genes in these group of organisms (Rodríguez-Martínez et al. 2011). Moreover, plasmid mediated quinolones resistance genes facilitate the chromosome-encoded quinolones resistance. The chromosome-encoded quinolones resistance is the most known mechanisms of quinolones resistance due to chromosomal mutations in the quinolone resistance-determining region of genes encoding DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) genes (Ni et al. 2016).

Plasmid-mediated quinolone resistance genes are also known to play an important role in resistance to aminoglycosides (Eftekhar 2015). This justifies our study results in that aminoglycoside (gentamicin and tobramycin) resistance rate of the ESBL positive isolates was very high when comparing with non ESBL producing strains. Other study also identified the co-existence of extended-spectrum beta-lactamase with *qnr*, *aac(6)-Ibcr* genes and genes encoding 16S rRNA methylases in the same ESBLs-producing strains to mediate multidrug resistance traits (Ho et al. 2016).

Similarly, the resistance rates for trimethoprim-sulfamethoxazole in ESBLs-producing strains in this study was very high. This is not surprising for ESBL positive organisms and it was well described in previous study (Wang et al. 2019). This indicates the coexistence of trimethoprim-sulfamethoxazole associated resistant genes such as *sul1* and *sul2* encoded on ESBL plasmids which facilitate the transmissions of these resistance determinants (Kozak et al. 2009).

ESBL positive isolates tested in this study showed relatively lower resistance to Nitrofurantoin, a relatively active agent against both ESBL positive and ESBL negative isolates. Similar study results were

reported from Kenya (Maina et al. 2013). This may be due to low prescription of this drug by physicians and therefore, nitrofurantoin remains active for treatment of non-life-threatening urinary tract infections (Maina et al. 2013).

Carbapenems, such as imipenem, meropenem and ertapenem, are the most effective antimicrobials for the treatment of infections caused by ESBL producing *Enterobacteriaceae* (Barbier et al. 2015). The rise in the prevalence of ESBL producing organisms directly associated with the increase in consumption of carbapenems led to the emergency of resistant organisms to this last generation antimicrobial agents which in turn limit the treatment option for infections caused by ESBL producing organisms (Barbier et al. 2015). In the current study, we report high prevalence of carbapenems resistant in ESBL positive isolates. This may indicate that the use of carbapenems for treatment bacterial infections might be widely spread in the country.

Conclusion

The prevalence of ESBL producing *Enterobacteriaceae* was very high in this study. *Enterobacteriaceae* capable of producing ESBL are public health threat not only because of the production the enzymes ESBL that hydrolyze penicillin and extended-spectrum cephalosporins, but also the multidrug resistance traits they exhibit to most clinically available antimicrobial agents as was evident in this study. Our study also showed a concerning level of carbapenems resistance, especially in ESBL positive isolates. Therefore, the impact of ESBL producing organisms on the treatment outcome of bacterial infection diseases in Ethiopia is potentially very high. Regular monitoring and strengthening of the national antimicrobial surveillance system and antimicrobial stewardship at health care facilities are very essential. Moreover, nationwide studies, involving molecular characterization of the isolates is needed to determine the epidemiology of ESBL related infections and the associated clinical burden.

Conflict of Interest: Authors declared no conflict of interest

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