Extended-spectrum Beta-lactamase and AmpC Beta-lactamase producing gram negative bacilli isolated from clinical specimens at International Clinical Laboratories, Addis Ababa, Ethiopia

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Abstract

Abstract Background: For Gram negative pathogens, production of beta-lactamase (β-lactamases) enzymes are the main mechanisms of resistance for most antimicrobial drugs. Of these β-lactamases, Extended spectrum Beta-lactamases (ESBL) and AmpC β-lactamases (AmpC) are the commonest enzymes produced by gram negative bacilli which are main mechanisms for the resistance all generations of cephalosporins. This study was therefore, aimed to assess the magnitude of ESBL and AmpC producing gram negative bacilli (GNB) isolated from clinical specimens at International clinical Laboratories, Addis Ababa, Ethiopia. Methods: A cross sectional study was conducted between January to May 2018. From different clinical specimens, 338 GNB were isolated. Bacterial species identification, antimicrobial susceptibility testing and β-lactamases screening were performed using phoenix automated system (BD phoenix 100). Cefoxitin resistant bacteria were confirmed for AmpC β-lactamases production by AmpC confirmatory disc. ESBLs production was confirmed using a combination disc method. Data were analyzed using SPSS version 20 software. Results: The predominant GNB was E. coli 66.0% (224/338) followed by K. pneumoniae 12.1% (41/338). The overall magnitude of ESBL producing GNB was 38.8% (131/338) while AmpC β-lactamases producing GNB was 2.4% (8/338). The majority of ESBLs and AmpC β-lactamases producing GNB were isolated from urine specimen 47.5% (116/338). Ampicillin (75.4%), amoxicillin with clavulanic acid (64.0%) and sulfamethoxazole-trimethoprim (55.6%) were most common resistance antibiotics. Multidrug resistance (MDR) level was 73.7% (249/338). Of ESBLs and AmpC β-lactamases producing GNB, 99.3% were MDR (P < 0.05). Conclusion: The magnitude of ESBL and AmpC β-lactamases producing GNB were significantly high which is worrying that needs intervention to minimize further occurrence and spread of such GNB. The MDR level was high which suggests continuous monitoring & reviewing of antimicrobial policy in
Background

Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are among the most important causes of nosocomial and community acquired bacterial infections in humans. Resistance to antimicrobial agents in such GNB has become important public health concern [1, 2]. The most commonly used antibiotics against infections caused by such MDR gram negative bacilli are β-lactam antibiotics. However, resistance for these β-lactam antibiotics due to the production of β-lactamases are becoming a worldwide threat. These can be developed when bacterial gene mutate continuously in response to overuse or misuse of β-lactam antibiotics [3-5]. Though there are more than 1000 β-lactamase enzymes, ESBL and AmpC β-lactamases are the most common types of beta-lactamases produced by GNB which are usually multidrug resistant bacteria [6, 7].

Extended-spectrum beta-lactamases hydrolyze beta-lactam class of antibiotic such as cephalosporins, penicillins, aztreonam and related oxyimino beta-lactams but not the cephemcins (cefoxitin) or carbapenems, and are inhibited by β-lactamase inhibitors such as clavulanic acid [8]. ESBLs are most commonly produced by *Klebsiella spp.* and *E. coli*. However, *Enterobacter*, *Salmonella*, *Proteus*, *Citrobacter*, *Morganella*, *Serratia*, *Shigella*, and *Pseudomonas* are also ESBL producers [9].

In addition to being resistant to β-lactam antibiotics, ESBL producing isolates are resistant to other classes of drugs such as aminoglycosides, fluoroquinolones and sulfonamides [10].

On the other hand, the Class C β-lactamases (AmpC) are clinically significant because they may confer resistance to penicillins, cephalosporins, cephemcins and monobactams. In
contrast to ESBL, AmpC β-lactamase activity is not affected by the ESBL inhibitor [11].

AmpC β-lactamase can be chromosomal or plasmid mediated while chromosomally mediated AmpC β-lactamase are found in Serratia, Pseudomonas, Acinetobacter, Citrobacter and Enterobacter spp. Plasmid mediated AmpC β-lactamase are commonly seen in most Enterobacteriaceae including E. coli, K. pneumoniae, Salmonella spp., Citrobacter freundii, and Proteus mirabilis [12, 13].

More importantly, Amp-C producing organisms act as hidden reservoir for ESBLs. Therefore, co-existence of these enzymes further complicates the treatment as Amp-C β-lactams may mask the recognition of ESBLs [2].

These ESBLs and AmpC β-lactamases production can be detected using different methods like conventional and automated techniques [10]. However, rapid and accurate detection of β-lactamase producing bacteria will play an important role for infection control and prevention of treatment failure. Therefore, one way to shorten the time consumed by microbiological analyses is use of automation system. Automation in microbiology laboratories such as Phoenix 100, Vitek-2, and MicroScan WalkAway 96 Plus are used for rapid identification of bacterial species and antimicrobial susceptibility testing [14, 15].

Gram negative bacilli that produce these enzymes causes multiple antibiotic resistances resulting for treatment failure, which in turn increase morbidity and mortality. Thus accurate and timely detection of ESBLs enzymes and AmpC β-lactamase is important for effective treatment of infections caused by such bacteria as well as for the prevention and control of the spread and further occurrence of drug resistance due to ESBLs and AmpC production. Hence, the present study aimed to determine the magnitude of ESBL and AmpC producing GNB isolated from clinical specimens at International clinical Laboratories, Addis Ababa, Ethiopia.

Methods
Study Area

The study was conducted at International Clinical Laboratories (ICL), Addis Ababa, Ethiopia. ICL was established in 2004. This laboratory has 14 Patient Service Centers (PSC's) and main laboratory located in Kera around old Bulgaria Mazoria, Addis Ababa. ICL is accredited by joint commission of international (JCI). Among different departments microbiology department is the one of the main department that received clinical specimens for microbiological analysis from different governmental and private hospital in Addis Ababa. It is equipped with automated machines including Phoenix automated system which is used for bacterial identification and antimicrobial susceptibility testing.

Study Design, Study Period and data collection

A cross sectional study was conducted from January to May, 2018. A total of 338 GNB isolates were collected from different clinical samples using convenient sampling method. The sample size was calculated based on single-population proportion using a previous study done in Ethiopia [16]. The socio-demographic data of patients was recorded using data collection sheet from the request form. The GNB isolate, the types of β-lactamases and the antibiotics susceptibility pattern of the isolate were also recorded using a separate data collection sheet.

Isolation and identification of GNB

All GNB isolates were recovered from various clinical specimens such as urine, pus, body fluids, sputum, stool, ear and eye discharge. The specimens were inoculated and incubated at 37°C for 18-24 hours on 5% sheep blood agar, XLD agar and MacConkey agar plates (All media were Oxoid Ltd, UK). The recovered colonies were characterized by colony appearance and gram stain. The significant growth colonies were examined morphologically for size, consistency, shape and ability to ferment lactose. Bacterial species identification was done using phoenix system (BD Diagnostic Systems, Oxford,
The turbidity 0.5 McFarland standards inoculum density of bacteria suspension was prepared from pure colony grown on primary isolation media and inoculated to the appropriate phoenix panel [17].

**Antimicrobial Susceptibility Test**

Antimicrobial susceptibility testing for 17 antimicrobials namely ceftazidime, cefotaxime, cefuroxime, ciprofloxacin, ceftriaxone, cefepime, amoxicillin with clavulanic acid, amikacin, aztreonam, ertapenem, cefoxitin, gentamicin, imipenem, meropenem, ampicillin, sulfametoxazole-trimethoprim, piperacillin/ tazobactam was performed using Phoenix AST panel (AST-N94) that apply microdilution method. The Phoenix machine has also screening test for ESBL and AmpC β-lactamases production among GNB [17].

**Screening for ESBL**

According to the CLSI guidelines, isolates that showed an MIC ≥2µg/ml for ceftazidime and/or for cefotaxime were considered as suspicious for ESBL productions [18].

**Confirmation of ESBL**

A disc of ceftazidime (30 µg) and cefotaxime (30 µg) alone and in combination with clavulanic acid (30 µg/10 µg) and cefotaxime (30 µg) + clavulanic acid (30 µg/10 µg) were placed at a distance of 25 mm apart on a Muller Hinton agar plate inoculated with bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight (18 – 24 hrs.) at 37°C. An increase in the inhibition zone diameter of ≥5 mm for a combination disc versus ceftazidime or cefotaxime disk alone was confirmed as ESBL producing bacilli according to CLSI (2018) guidelines [18].

**Screening of AmpC beta-lactamase**

Guided with EUCAST, isolates showing reduced susceptibility to cefoxitin (MIC >8 µg/ml) were identified as potential AmpC β-lactamase producer. In addition, all ESBLs suspicious isolates were screened for confirmation [19].
Confirmation of AmpC beta-lactamase

All the cefoxitin non susceptible isolates and ESBLs suspicious isolates were checked for the presence of AmpC β-lactamase using disc diffusion tablets Neo-Sensitabs (ROSCO, Taastrupgaardsvej 30, DK-2630 Taastrup, Denmark) one tablet with cefotaxime, one with ceftazidime and two tablets of the cephalosporins combined with cloxacillin (AmpC inhibitor). An increase in the inhibition zone diameter of ≥5 mm for a combination disc versus ceftazidime or cefotaxime disk alone was confirmed as AmpC β-lactamase producing GNB [20].

Data Quality Assurance

The prepared culture media was checked for sterility by incubating the five percent of prepared media for overnight and observe for the presence of any growth. Quality control for phoenix machine and the prepared media were performed using ATCC control strains. For ESBL K. pneumoniae ATCC 700603 (positive control) and E. coli ATCC 25922 (negative control) strains were used. Enterobacter cloacae (ATCC BAA 1143) and E. coli (ATCC 25922) used as positive and negative QC strains for AmpC β-lactamase producing GNB respectively. Before applying the 20% glycerol TSB for storage, it was QC tested for growth of E. coli ATCC 25922 standard strains. Data was collected using worksheet, entered and analyzed using SPSS version 20 software.

Data Analysis and Interpretation

Data were analyzed using SPSS version 20 software. The descriptive summaries were presented with tables and graphs. Proportions and actual number of AmpC and ESBLs producing GNB isolates were used to describe frequency of categorical variables.

Continuous variables were described by mean and standard deviation.

Ethical considerations

The study was conducted after obtaining ethical clearance from the department research
and ethical review committee of the department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences; Addis Ababa University. Additionally, an official permission letter was obtained from the study site.

Results

Socio-demographic characteristics

A total of 338 GNB isolates were identified from various clinical specimens of different sources of infections during the study period. These isolates were recovered from clinical specimens arrived to ICL from government (151) and private (187) hospitals. The majority of the GNB were isolated from urine 72.2% (244/338) followed by pus 18.6% (63/338) (Table 1).

Among the total isolates, 58.3% (n= 197/338) were from females and 41.7% (n=141/338) were collected from males with females to males ratio 1.39:1. From all GNB isolated from females, *E. coli* (68.5%) and *K. pneumoniae* (14.2%) were the most frequently isolated bacteria. Similarly among males *E. coli* (63.1%) and *K. pneumoniae* (9.2%) were predominant isolates. The majority of isolates 86 (25.4%) were obtained from patients above 61 years of age and the mean age was 43.9 years and standard deviation 21.8.

Frequency of gram negative bacilli isolates

Among all 338 GNB isolates, *E. coli* with 66.3% (224/338) was the most frequent isolates followed by *K. pneumoniae* with 12.1% (41/338). Out of the 224 *E. coli*, 84.4% (189/224) were isolated from urine and 10.7% (24/224) were from pus specimens. From non-fermenters *pseudomonas* spp. were obtained mostly from pus with 41.2% (7/17), followed by discharge 29.4% (5/17) and urine 23.5% (4/17) (Table 1).

Antibiotics Resistance pattern of gram negative bacilli

For all isolates the drug susceptibility testing was done by broth microdilution method. Highest resistance level was recorded to ampicillin (75.4%) followed by amoxicillin with
clavulanic acid (64.0%), sulfamethoxazole-trimethoprim (55.6%), cefuroxime (48.2%) and cefotaxime (47.0%). *E. coli* showed highest resistance to ampicillin (77.2%) followed by amoxicillin with clavulanic acid (67.9%). In *K. pneumoniae* the highest level of resistance was observed against ampicillin (100%), amoxicillin with clavulanic acid (73.2%), sulfamethoxazole-trimethoprim (70.7%), cefuroxime (65.9%) and azthreonam (63.4%); while no resistance observed to amikacin (0.0%) and low resistance level to meropenem and ertapenem (9.8%), imipenem (12.2%) (*Table 2*).

**Multi-drug resistance pattern of gram negative bacilli**

Among the total isolates (n=338) multi-drug resistance (non-susceptible to at least 3 antibiotics belonging to different antibiotics categories) level were recorded in 249 (73.7%) of all bacterial isolates. Relatively higher rate of MDR was seen among *Enterobacter spp.*, *Citrobacter spp.*, *Acinetobacter spp.*, *K. pneumoniae* and *E. coli* accounting average resistance of 90.9%, 90.0%, 88.9%, 82.9% and 69.6% respectively. Surprisingly, the average MDR rate of *Pseudomonas spp.* was found to be 100% and *K. oxytoca* & *K. ozenae* were not MDR isolate. The most effective antibiotics for MDR were amikacin, meropenem and imipenem with sensitivity of 97.1%, 96.3% and 95.5% respectively (*Table 3*).

**Magnitude of ESBLs Producing gram negative bacilli**

Among 135 ESBL suspected GNB with MIC ≥2µg/ml for ceftazidime and/or for cefotaxime using phoenix system, 38.8% (131/338) were confirmed as ESBLs producing GNB using combination disk test.

The higher percentage of ESBL producing GNB was recorded in *K. pneumoniae* 56.1% (n=23/41) followed by *E. coli*, *E. cloacae* and *Citrobacter Spp.* with 44.6% (100/224), 36.4% (4/11) and 10.0% (1/10) respectively (*Figure 1*). Urine constitute majority of ESBL producing GNB and the proportion of ESBLs was significantly high among isolates from
adult patients > 61 years of age (P < 0.05).

**Magnitude of AmpC producing gram negative bacilli**

The overall magnitude of AmpC beta-lactamase producing GNB was 2.4% (8/338). *E. coli* 1.2% (4/338), *K. pneumoniae* 0.9% (3/338) and *Citrobacter Spp.* 0.3% (1/338) were AmpC producers among other GNB isolates.

The distribution of AmpC β-lactamase producers varied among different species of GNB. The highest frequency of AmpC β-lactamase production was observed among *K. pneumoniae* 7.3% (3/41) followed by *E. coli* 1.8% (4/224).

From 80 cefoxitin resistant (MIC >16 µg/ml) isolates, 10% (8/80) were confirmed as AmpC β-lactamase producing GNB. All AmpC β-lactamase producing GNB were isolated only in urine specimen 3.3% (8/244). This enzyme was more prevalent (40.1%) among age group above sixty one years old.

In general, MDR in this study was 73.7% (249/338). Of these ESBL account 38.2% and AmpC was 2.4%. From these β-lactamase producing GNB, the most frequently observed bacteria were *K. pneumoniae, E. coli* and *Enterobacter spp.* (Figure 1).

**Distribution of ESBL and AmpC β-lactamase producing gram negative bacilli with their MDR level among different specimens**

Among all GNB, 41.4% (140/338) were beta-lactamase producers & 73.7% (n=249/338) were MDR. From the specimen we analyzed ESBL and/or AmpC producing GNB 44.7% (109/244) was found predominantly in urine. On the other hand maximum number of MDR was found in wound 81.0% (51/249) (Figure 2). Of ESBL and/or AmpC producing GNB, 99.3% (n=138/139) were MDR (P < 0.05). The rest 0.7% (n=1/139) non-β-lactamases producing GNB were MDR due to other mechanism of resistance. Being β-lactamases producer has statistically significant association with MDR (P=0.001).

**Antibiotics sensitivity pattern of ESBLs and AmpC producing gram negative**
bacilli against different class of antibiotics

Overall non ESBL and/or AmpC producing GNB were more sensitive to the antibiotics than ESBL and/or AmpC producers. The most active drugs for ESBL-producing isolates were amikacin (100), imipenem (99.2), ertapenem (98.5), meropenem (97.7) and pipracillin/tazobactam (87.8). On the other side, AmpC producing GNB showed high sensitivity to amikacin, meropenem, imipenem, ertapenem and pipracillin/tazobactam with susceptibility results of 100%, 100%, 87.5%, 87.4% and 87.2% respectively. Moreover, gentamycin and ciprofloxacin can be used as alternative treatment for these enzyme producing isolates.

Discussions

Antibiotics Resistance pattern of gram negative bacilli

The level of antimicrobial resistance of identified gram negative isolates was ranging from 0%-75.4%. The present study showed that there was higher resistance to ampicillin (75.4%), followed by amoxicillin with clavulanic acid (64.0%), sulfamethoxazole-trimethoprim (55.6%), aztreonam and cefuroxime (48.8%), cefotaxime (47.0%), cefepime (45.6%), ceftriaxone (44.9%), ceftazidime (44.1%). There were also significant level of resistance to ciprofloxacin (40.2%) and gentamycin (21.3). Comparable result were reported in Ethiopia such as in Gondar: ampicillin (84.6%) and sulfamethoxazole-trimethoprim (79.5%) and gentamicin (35.9%) [16], Debre Markos: ampicillin (70.4%), amoxicillin with clavulanic acid (58.8%), sulfamethoxazole-trimethoprim (53.1%) [21]. However, the resistance level was lower than a study conducted in Tanzania: ampicillin (100%), amoxicillin with clavulanic acid (98.7%), sulfamethoxazole-trimethoprim (95.2%), ceftazidime (74.0%) [22], Southeast Iran: sulfamethoxazole-trimethoprim (93.8%) and amoxicillin with clavulanic acid (91.4%) [23] and in Sierra Leone: sulfamethoxazole-trimethoprim (91.4%), gentamycin (72.9%) [24]. The possible reason for this difference
might be due to indiscriminate use of antibiotics, patient condition and majority of bacteria in these countries were β-lactamases producing GNB.

**Multi drug resistance pattern of gram negative bacilli**

In the present study, the overall magnitude of MDR among all GNB isolate was 73.7%. There were also similar findings from studies conducted in Gondar (68.0%) [25], Dessie (74.6%) [26], Debre Markos: (72.2%) [21] and Nepal (64.0%) [27]. However, our result was lower than studies done in Sierra Leone (85.7 %) [24], Gondar (87.4%) [16], Bahir Dar (93.1%) [28], Nepal (96.8%) [29]. The difference in magnitude of MDR isolates might be due to patient condition, definition for MDR and empirical treatment trend. In addition, our result was higher when compared to a previous study done in Jimma (59.3%) [30], Nepal (54.2%) [27], another study in Nepal by Lamichhane et al [31] reported (33.14%). However, the increased proportion of MDR seen in this study was considered as alarming because only a few treatment options remain for infections. Therefore, implementing strong infection control strategies is required to reduce MDR burden.

The present study showed that *Pseudomonas spp.* (100%), *Enterobacter spp.* (90.0%) and *Citrobacter spp* (90.0%) were found to be the principal MDR isolates which agreed with a study done in Jimma: *Citrobacter spp.* 100% [30], in Nepal: *Enterobacter spp.* 71.4% [31]. Different studies showed different pathogens as a major MDR isolates, in Gondar: *K. pneumoniae* (95.6%) and *E. coli* (92.9%) [16], Sierra Leone: *K. pneumoniae* (73.3%) and *E. coli* (61.5%) [23], Nepal: *K. pneumoniae* 100% & *E. coli* 95.5% [29] were found to be the predominant MDR isolates. These pathogens are the most commonly found in both hospital and community acquired infections. In addition, these bacteria are resistance to multiple groups of antimicrobial agents, this makes treatment difficult [27].

**Magnitude of ESBLs producing gram negative bacilli**

The overall magnitude of ESBLs producing GNB in the present study was 38.8%, which was
in agreement with study reported in Harrer 33.3% [32], Nepal 34.5% [27], Spain 42.8% [33], India 44.0% [34]. ESBLs producing organisms are the major cause of treatment failure, reduced rate of clinical and microbiological responses, longer hospital stay and increased cost of hospital [35].

However, the magnitude of ESBLs production among GNB in our study was lower than studies done in Addis Ababa 57.7% [36], Bahir Dar 57.6% [28], North West Nigeria 58.0% [37], Southwestern Uganda 89% [38] and Southeast Iran 53.8% [23]. This wide variation might be due to differences in study population, type of specimen, sample size, the extent of antibiotic use and mainly the methodology used.

To the contrary, our finding was higher than studies reported in Adama 25.0% [39], Nigeria 15.8% [40], Nepal 26.8 % [29] and Italy (6.3%) [41]. This indicated that ESBL-producing organisms are increasing from time to time.

In the current study, the predominant ESBLs producing GNB was K. pneumoniae (56.1%) than E. coli (43.8%). This finding was supported by previous studies done in Addis Ababa, K. pneumoniae (78.6%) and E. coli (52.2%) [36], Bahir Dar: K. pneumoniae (69.8%) and E. coli (58.2%) [28], North West Nigeria: K. pneumoniae (62.9%) and E. coli (54.2%) [37], Southwestern Uganda: K. pneumoniae (52%) and E. coli (44%) [38], Nairobi: K. pneumoniae (78.8%) and E. coli (60.7%) [42], Uganda: K. pneumoniae (72.7 %) and E. coli (58.1% [43]. On the other side, our finding was in contrary to study conducted in Sri Lanka: E. coli (86.8%) and K. pneumoniae (13.1%) [44], India: E. coli (50.14%) and K. pneumoniae (48.3%) [45] in which E. coli was the predominant ESBLs producer than K. pneumoniae.

**Magnitude of AmpC producing gram negative bacilli**

AmpC β-lactamases producing GNB have been responsible for several nosocomial outbreaks and high rate of treatment failure [46].
In the present study, from the total specimens, 2.4% (8/338) AmpC producing GNB were isolated. This finding was in line with the study done in Iran 1.5% [47], Greek 2.6% [48], India (8%) [49]. However, it was lower than the finding in Nigeria (15.2%) [46], Spain (14.2%) [33], India (37%) [34]. Differences in finding between these countries might be related to detection methods, study participants, geographic area and AmpC genes prevalence difference.

In present study, *K. pneumoniae* 7.3% (3/41) followed by *E. coli* 2.2% (5/224) were the principal AmpC producing pathogen which was in line with study done in Turkey: *K. pneumoniae* 3.6% [50] and in Spain: *K. pneumoniae* 5.8% were common AmpC producer [33]. This might be due to the fact that plasmid mediated AmpC β-lactamases are seen in *Enterobacteriaceae* and these genes are easily transferable horizontally [13].

The current study also demonstrated co-existence of ESBL and AmpC enzymes in five isolates 3.6% (5/139). This finding was concordant with study conducted in Nigeria (6.04%) [40], South India (4.4%) [2], India (9.9%) [9] Simultaneous production of ESBL and AmpC enzymes in a bacterium causes false negative confirmatory test for ESBL production. In other word, existence of plasmid mediated AmpC beta-lactamase enzyme can mask the presence of ESBL [51]. Therefore, simultaneous detection of these enzymes helps to prevent missing of ESBL.

**Antibiotics susceptibility pattern of ESBLs and AmpC producing gram negative bacilli**

In present study, the highest rate of susceptibility of ESBLs producing isolates were found toward amikacin (100%) followed by imipenem (99.2%), meropenem (97.7%), ertapenem (98.5%), piperacillin/tazobactam (87.0%) and cefoxitine (84.0%). This finding was in harmony with the findings of other studies conducted in Nepal: imipenem (100%), piperacillin/tazobactam (93.3 %), and amikacin (91.8%) [27], Sri Lanka: meropenem
(95%), imipenem (73.7%) and amikacin (60.6%) [44], India: imipenem (100%),
piperacillin/tazobactam (89.3%), meropenem (87.5%), and amikacin (83.9%) [45], Ghana: 100% sensitive to meropenem [52], and study in India [34] showed 98.0% isolates were sensitive to imipenem.

The present study indicated that ESBL producers had significant levels of resistance to third generation cephalosporin, penicillin & sulfonamide. Similar finding observed in Adama, Uganda and Ghana [39, 43, 52]. Highest levels of resistance to ampicillin (99.2%), ceftazidime (98.5%), ceftriaxone (98.5%), amoxicillin with clavulanic acid (98.0%) and sulfamethoxazole-trimethoprim (81.0%) observed in this study was in agreement with the study done in Nepal: amoxicillin with clavulanic acid (100%), sulfamethoxazole-trimethoprim (59%) [27], India: ceftazidime (97%), ceftriaxone (76%) [53], another study in India done by Shashwati et al, amoxicillin with clavulanic acid (89.3%), sulfamethoxazole-trimethoprim (94.6%) [45].

Most of the AmpC producing GNB were resistant to the commonly used antibiotics as seen in different studies [51, 54, 40]. In our finding, among cefoxitin resistance isolates, 10% (8/80) were AmpC producers which was in agreement with the study done in Iran, 5.1% [47], Turkey, 8.7% [50] but lower than the finding in India, 37.0% [53]. The possible reasons for cefoxitin resistance in the absence of AmpC production might be due to resistance mechanism like loose of permeability of porins and other [55].

The present study showed that AmpC producers were 100% sensitive to imipenem and meropenem (100%) but highly resistance against ampicillin (100%), amoxicillin with clavulanic acid (97.0%), ceftriaxone (89.0%), sulfamethoxazole-trimethoprim (75.0%), ceftazidime (68.0%), and cefepime (50.0%). This finding was in line with study conducted in Turkey showed AmpC producers were 100% sensitive to imipenem and meropenem, 92.0% to amikacin and 82.0% resistance against amoxicillin with clavulanic acid, 68.0% to
ceftazidime and 49.0% to cefepime [50], Likewise, in Nigeria: AmpC producers were resistant to amoxicillin with clavulanic acid (77.9%), ceftazidime (75.0%) [46], India: resistance level to amoxicillin with clavulanic acid was (95.9%), to sulfamethoxazole-trimethoprim (82.9%), and to ceftazidime (87.1%) [53]. AmpC producers seem susceptible to cephalosporins in-vitro but when cephalosporins are used in vivo, they result in failure of treatment [56, 57]. Therefore, cephalosporins could not be useful in treating infections caused by AmpC producing bacteria.

**Distribution of ESBL and AmpC beta-lactamase producing gram negative bacilli in different specimens**

The present study showed that ESBL and/or AmpC producing GNB were predominantly found in urine 44.7% (109/244) followed by in pus 34.9% (22/63). This might be due to the larger number of urine samples were included in this study. In this study maximum ESBL producers 41.8% (102/244) were found in urine. Fairly similar finding was also reported in different countries such as central India (52.28%) [45], Northwest Nigeria (63.5%) [37], Uganda 64.9 % [43], Ghana 66.7 % [52], Sierra Leone 64.3 % [24], India 52.3% [45] and India by Tewari et al, 35% [53]. However, study done in Adama, showed that major source of ESBLs producing GNB (53.0%) were isolated from pus [39].The difference might be due to proportion of urine specimen difference, study participant difference and risk factors.

All AmpC producing pathogen in our study was isolated from urine 3.3% (8/244). This finding was in close agreement with study conducted in Turkey and Nigeria [50, 57] but it was not supported by Ogefere et al [46] in Nigeria where isolates from sputum (50.0%) were the predominant producers of AmpC β-lactamase. This indicated that the prevalence of AmpC β-lactamase may differs significantly among bacteria recovered from different clinical specimens [34].

**Strength of the study**
This is the first study done on the magnitude of ESBL and AmpC β-lactamase producing GNB in Ethiopia using automation for screening and CLSI recommended conventional methods for confirmation of these β-lactamase producing GNB. The study tried to show the antimicrobial susceptibility pattern of ESBL and AmpC β-lactamase producing GNB.

Limitation of the Study
Magnitude of ESBLs and AmpC producing GNB from blood culture were not addressed due to the downtime of the Bactech 9050 machine during the study period.
Since specimens were collected from different hospitals and arrived to ICL, we are unable to realize possible risk factors and the outcome of the patients infected with these β-lactamase producing bacteria.

Conclusion
In this study, higher magnitude of ESBLs and AmpC β-lactamases production among GNB was found. The majority of these β-lactamases producing isolates were obtained from urine specimen. K. pneumoniae and E. coli was the most frequent ESBLs and AmpC β-lactamase producing GNB. These ESBLs and AmpC β-lactamases producing GNB showed higher level of MDR. The most effective antibiotics for treatment of these groups of β-lactamases producing GNB were amikacin, meropenem and imipenem. The progress of ESBLs and AmpC β-lactamase requires strengthening of antimicrobial resistance surveillance system and effective antibiotic policy like antibiotic restriction, combination therapy and infection control programs combined with good medical practices. Large scale researches that can assess wide geographical area with more representative sample need to be done in country.

List Of Abbreviations
CLSI: Clinical and Laboratory Standards Institute, ICL: International Clinical Laboratories, ESBLs: Extended-spectrum beta-lactamases, AmpC: AmpC β-lactamase, MDR: Multidrug Resistant

Declarations
Ethics approval
The study was approved by the department of research and ethics review committee of the Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences; Addis Ababa University. Permission was obtained from international clinical laboratories to collect data and use the necessary materials.

Consent for publication
Not applicable

Availability of data and material
The present study data can be obtained from the corresponding author when requested reasonably.

Competing Interest
The authors declare that they have no conflicts of interest in this research work

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Costs of Antibiotics disks and media were covered by the principal investigator. However, the other supplies were covered by ICL and AAU.

Authors' contributions
SG: Study design, analysis, data collection, laboratory work and interpretation of result as well as writing of manuscript; DS: Supervise in designing, laboratory work, analysis and review manuscript. SK, KD and MH: Advising and reviewing the manuscript. All authors read and approved the final manuscript.

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Tables
Table 1: Distribution of GNB isolate against demographic characteristics and specimen types

| Variables (Number) | E.coli | K.pneumoniae | K.oxytoca | K.ozonea | Pseudomonas Spps. | Enterob Spps. |
|--------------------|--------|--------------|-----------|----------|-------------------|---------------|
| Sex                |        |              |           |          |                   |               |
| Male(141)          | 89(63.1) | 13(9.2)     | 1(0.7)    | 0(0.00)  | 5(3.5)            | 8(13)         |
| Female(197)        | 135(68.5) | 28(14.2)    | 3(1.5)    | 1(0.5)   | 12(6.1)           | 3(1)          |
| Age group          |        |              |           |          |                   |               |
| ≤15(35)            | 18(51.4) | 4(11.4)     | 3(8.6)    | 0(0.0)   | 2(5.7)            | 0(0)          |
| 16-<32(73)         | 52(71.2) | 6(8.2)      | 1(1.4)    | 1(1.4)   | 2(2.7)            | 4(5.1)        |
| 32-<46(74)         | 49(66.2) | 9(12.2)     | 0(0.0)    | 0(0.0)   | 3(4.1)            | 4(5.1)        |
| 46-<61(70)         | 43(61.4) | 10(14.3)    | 0(0.0)    | 0(0.0)   | 7(10.0)           | 1(1)          |
| ≥61(86)            | 62(72.1) | 12(14.0)    | 0(0.0)    | 0(0.0)   | 3(3.5)            | 2(2.4)        |
| Types of Specimen  |        |              |           |          |                   |               |
| Urine(244)         | 189(77.5) | 23(9.4)     | 1(0.4)    | 0(0.0)   | 4(1.6)            | 5(2)          |
| Pus (63)           | 24(38.1) | 11(17.5)    | 2(3.2)    | 0(0.0)   | 7(11.1)           | 6(9.5)        |
| Body fluid(10)     | 7(70.0)  | 2(20.0)     | 0(0.0)    | 0(0.0)   | 0(0.0)            | 0(0)          |
| Discharge(13)      | 1(7.7)   | 3(23.1)     | 1(7.7)    | 1(100)   | 5(38.5)           | 0(0)          |
| Sputum(6)          | 3(50.0)  | 2(33.3)     | 0(0.0)    | 0(0.0)   | 1(16.7)           | 0(0)          |
| Stool(2)           | 0(0.0)   | 0(0.0)      | 0(0.0)    | 0(0.0)   | 0(0.0)            | 0(0)          |
| Total (N=338)      | 224(66.3) | 41(12.1)    | 4(1.2)    | 1(0.3)   | 17(5.0)           | 11(3.3)       |

Note: *other isolates are Salmonella spp., Providential spp., M.morganii, Serratia spp.

Table 2: Antimicrobial resistance pattern of GNB isolated from different clinical specimens
| Isolates (N)            | CRO  | CAZ  | FEP  | CTX  | CXM  | FOX  | MER  | IMP  | ETP  | SXT  | Note |
|------------------------|------|------|------|------|------|------|------|------|------|------|-------|
| *E. coli* (n=224)      | 110  | 108  | 110  | 114  | 115  | 20   | 2    | 1    | 5    | 135  | TZP: pipracillin/tazobactem, GM: gentamicin, AMP: ampicillin, MEM: meropenem, IMP: imipenem, ETP: ertapenem, SXT: trimethoprim–sulfamethoxazole, FOX: Cefoxitin, CRO: ceftriaxone, CAZ: ceftazidime, ATM: azthrenam AN: amikacin, AMC: amoxicillin–clavulanic acid, FEP: Cefepime. |
|                        | (49.1)| (48.2)| (49.1)| (50.9)| (51.3)| (8.9)| (0.9)| (0.4)| (2.2)| (60.3)|       |
| *K. pneumoniae* (n=41) | 26   | 25   | 25   | 26   | 27   | 7    | 4    | 5    | 4    | 29   | (60.3) |
|                        | (63.4)| (61.0)| (61.0)| (63.4)| (65.9)| (17.1)| (9.8)| (12.2)| (9.8)|       |
| *K. oxytoca* (n=3)     | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | (0.0) |
|                        | (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)|       |
| *K. ozonea* (n=1)      | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | (0.0) |
|                        | (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)| (0.0)|       |
| *Pseudomonas Spps.* (n=17) | NA  | 4    | 4    | NA   | NA   | 1    | 0    | NA   | NA   |      |       |
|                        |     | (23.5)| (23.5)|       |       | (5.9)| (0.0)|       |       |       |       |
| *Enterobacter Spp.* (n=11) | 8   | 5    | 7    | 8    | 8    | 9    | 0    | 0    | 1    | 8    | (72.7) |
|                        | (72.7)| (45.5)| (63.6)| (72.7)| (72.7)| (81.8)| (0.0)| (0.0)| (9.0)|       |
| *Citrobacter Spps.* (n=10) | 2   | 2    | 1    | 5    | 4    | 0    | 2    | 0    | 6    | (60.0)|       |
|                        | (20.0)| (20.0)| (10.0)| (50.0)| (40.0)| (0.0)| (20.0)| (0.0)|       |       |
| *Acinetobacter Spps.* (n=9) | NA  | 2    | 3    | NA   | NA   | 0    | 0    | NA   | NA   |      |       |
|                        |     | (22.2)| (33.3)|       |       | (0.0)| (0.0)|       |       |       |       |
| *P. mirabilis* (n=5)   | 1    | 0    | 1    | 1    | 2    | 1    | 1    | 0    | 2    | (40.0)|       |
|                        | (20.0)| (0.0)| (20.0)| (20.0)| (40.0)| (20.0)| (20.0)| (0.0)| (0.0)|       |
| *Shigella Spps.* (n=2) | 0    | 0    | 0    | 0    | NA   | 1    | 0    | 0    | 1    | (50.0)|       |
|                        | (0.0)| (0.0)| (0.0)| (0.0)|       | (50.0)| (0.0)| (0.0)| (0.0)|       |       |
| *Salmonella Spps.* (n=4) | 0    | NA   | NA   | 1    | NA   | 1    | 0    | 1    | 1    | (50.0)|       |
|                        | (0.0)|       |       | (50.0)|       | (50.0)| (0.0)| (0.0)| (0.0)|       |       |
| *M. morganii* (n=4)    | 3    | 2    | 3    | 3    | NA   | 1    | 1    | 1    | 3    | (75.0)|       |
|                        | (75.0)| (50.0)| (75.0)| (75.0)|       | (25.0)| (25.0)| (25.0)|       |       |
| *Providencia spps.* (n=6) | 1    | 0    | 0    | 2    | 4    | 1    | 0    | 1    | 2    | (33.3)|       |
|                        | (16.7)| (0.0)| (0.0)| (33.3)| (66.7)| (16.7)| (0.0)| (16.7)| (33.3)|       |       |
| *Serratia Spps.* (n=3) | 1    | 1    | 2    | 3    | 0    | 0    | 1    | (33.3)|       |       |
|                        | (33.3)| (33.3)| (33.3)| (100)| (66.7)| (66.7)| (0.0)| (33.3)|       |       |       |
| Total Resistance (n=338) | 152  | 149  | 154  | 159  | 163  | 49   | 9    | 11   | 14   | 188  |       |
|                        | (44.9)| (44.1)| (45.6)| (47.0)| (48.2)| (14.5)| (2.7)| (3.3)| (4.1)|       |       |

Table 3: Multidrug resistance pattern of GNB isolated from different clinical specimens
| Isolates (number) | R0  | R1  | R2  | R3  | R4  | R5  | R6  |
|------------------|-----|-----|-----|-----|-----|-----|-----|
| E. coli (224)    | 28(12.5) | 18(8.0) | 22(9.8) | 33(14.7) | 12(5.4) | 29(12.9) | 33(14.7) |
| K. pneumoniae (41) | 2(4.9) | 3(7.3) | 2(4.9) | 6(14.6) | 2(4.9) | 2(4.9) | 9(22.0) |
| K. oxytoca (4)   | 2(50.0) | 2(50) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| K. ozanea (1)    | 1(100) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| Pseudomonas Spp. (17) | 0(0.0) | 0(0.0) | 0(0.0) | 2(11.8) | 1(5.9) | 0(0.0) | 4(23.5) |
| Enterobacter Spp. (11) | 0(0.0) | 0(0.0) | 1(9.1) | 0(0.0) | 2(18.2) | 0(0.0) | 1(9.1) |
| Citrobacter Spp. (10) | 0(0.0) | 0(0.0) | 1(10.0) | 2(20.0) | 2(20.0) | 1(10.0) | 4(40.0) |
| Acinetobacter Spp. (9) | 0(0.0) | 0(0.0) | 1(11.1) | 3(33.3) | 1(11.1) | 1(11.1) | 1(11.1) |
| P. mirabilis (5) | 2(40.0) | 0(0.0) | 1(20.0) | 0(0.0) | 1(20.0) | 0(0.0) | 1(20.0) |
| Shigella Spp. (2) | 1(50.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 1(50.0) | 0(0.0) |
| Salmonella Spp. (2) | 1(50.0) | 1(50.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| M. morgani (4)  | 2(50.0) | 1(25.0) | 0(0.0) | 1(25.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| Prividentia Spp. (6) | 1(16.7) | 3(50.0) | 0(0.0) | 1(16.7) | 0(0.0) | 1(16.7) | 0(0.0) |
| Serratia Spp. (3) | 1(33.3) | 2(66.7) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| Total (n=338)    | 38(11.2) | 25(7.4) | 31(9.2) | 45(13.3) | 23(6.8) | 36(10.6) | 54(16.0) |

Note: R0: resistance to no antibiotics, R1-7: resistance to 1, 2, 3, 4, 5, 6, and 7 antibiotics respectively; MDR: resistance to three or more antibiotics from different classes

Figures
Distribution of ESBL and AmpC β-lactamases producing GNB and MDR level among GNB.

Note: Other isolates are *P. mirabilis, Shigella Spp, Salmonella spp, Providencia spp, M. morganii and Serratia spp.*

Figure 1
Figure 2

Distribution of ESBL, AmpC β-lactamase producing GNB and MDR isolate from different clinical specimens.