Occurrence and Anti-microbial Susceptibility Pattern of Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae in Waste Water Released from Governmental Hospital of Addis Ababa, Ethiopia

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Abstract

Background

Worldwide, come out and dissemination of Extended-spectrum beta-lactamases (ESBLs) producing Enterobacteriaceae has been warning the efficacy of antibiotics to treat an infection. Hospital wastewaters were a reservoir of such kind of resistant bacteria. Currently, the predominant antibiotics used for the treatment of hospitalized patients infected by Gram negative bacteria are the β-lactam antibiotics. So it is an important source to investigate the magnitude of ESBLs producing bacteria and their antimicrobial susceptibility pattern. The aim of this study was to determine the occurrence of ESBLs producing Enterobacteriaceae (ESBLs-pE) and their antibiotic susceptibility pattern in wastewater released from five governmental hospitals in Addis Ababa, Ethiopia.

Method

A cross-sectional study was carried out from April 1 to May 31, 2020. A total of 100 wastewaters were collected from five governmental hospitals in Addis Ababa using a grab-sampling technique. All Enterobacteriaceae were screened for ESBLs production using cefotaxime and ceftazidime as per 29th CLSI guideline. Each screen positive for ESBLs production was confirmed by the combination disk method (CDT) and their antibiotic susceptibility pattern were done using the Kirby-Bauer disk diffusion method on Muller Hinton agar (MHA). Data were entered and summarized using SPSS version 20 software.

Results

Of all Enterobacteriaceae, 48.3% were confirmed ESBLs-pE. The highest ratio of ESBLs-pE was observed in adult ward (66.7%) and laundry unit effluent (58.8%). The highest ESBL producers were E. coli (21.8%) and K. pneumoniae (4.8%). The most elevated resistance level of ESBL producer were observed to cefotaxime (95.8%) and amoxicillin/clavulenate (93%). 64% of tested Enterobacteriaceae isolates were multi drug resistant (MDR).

Conclusion

Higher magnitude of MDR and ESBLs-pE were present in the hospital wastewater. Majority of them were in adult ward and laundry unit effluents. The most frequent ESBLs-pE was among E. coli and K. pneumoniae. Hence, Consistent infection prevention and control procedures should be in practice at each ward/unit.

Introduction

Wastewater refers to water used by humans and loses its quality and usefulness that embrace waste liquid of domestic, agricultural, commercial sources, industries, and hospital sources. Every these anthropogenic activity generates wastewater as a result of physical, chemical and biological quality of water deterioration (1). The effluent from hospital contains a lot of drug-resistant pathogens, a larger form of chemicals, solvents, disinfectants and a lot of risky materials like pharmaceuticals and radionuclides than domestic sewerage (2). As a result of this hospital effluent contains antibiotic residues that are enough to kill susceptible bacteria and at the same time increases the number of resistant bacteria (3). The presence of antibiotic-resistant microorganism in effluent and sewage system line is a growing public health concern (4). Since one of the main ways of diffusion of pathogenic and/or antibiotic resistant microorganism is thru water, soil and air. This phenomenon results multi-drug resistant (MDR) microorganisms that have been revealed in different water sources including rivers, lakes, groundwater and drinking water (5-8). The discharge of resistant bacteria to the receiving aquatic environment will create public health impact through, carrying transmissible gene, by acting as a vector or reservoir of resistant gene (9, 10).

The members of the Enterobacteriaceae are gram-negative bacilli, which are usually resident in the gastrointestinal tract. Instance of such organisms consisting of E. coli, K. pneumoniae, Enterobacter cloacae, Citrobacter freundii and Proteus mirabilis. In patients hospitalized in intensive care units (ICUs), the Enterobacteriaceae holds for about one third of all cases of ICU-acquired pneumonia, one third of all cases of ICU-acquired urinary tract infection, and 10% to 15% of ICU-acquired bloodstream infections (11).

Currently, the dominant antibiotics used for treating hospitalized patients infected by Gram negative bacteria are the β-lactam antibiotics that inhibit transpeptidases taking part in bacterial cell wall synthesis. Sadly, these β-lactam antibiotics will be deactivated by β-lactamase enzymes (12). A persistent exposure of bacterial strains to a multitude of β-lactam antibiotics has evoked a dynamic, continuous production and mutation of beta lactamase in the bacteria resulting in the event of extended spectrum β-lactamases (ESBLs) inflicting resistant to broad spectrum β-lactam antibiotics (12-15). ESBLs are beta-lactamases that confer resistant to the penicillins; first, second, and third generation cephalosporins and monobactams by hydrolysis of these antimicrobials. Additionally, these enzymes are pent-up by beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. ESBLs are encoded on transferrable conjugative plasmids that facilitate widespread dissemination, not solely between the identical species of bacteria, but also across completely different species. Furthermore, these plasmids code for resistant to other classes of potent antimicrobial agents, in significantly, aminoglycosides and fluoroquinolones (16). Among the numerous ESBLs described in a variety of pathogens,CTX-M, TEM, and SHV types proved to be the most successful in terms of promiscuity and dissemination across various epidemiological niches (17).

The first ESBLs were isolated in the 1980’s (18, 19). ESBLs are isolated from a large type of Enterobacteriaceae (foremost in E. coli and K. pneumoniae), Pseudomonas aeruginosa and Capnocytophaga ochracea (20). The increasing prevalence of ESBL Enterobacteriaceae especially K. pneumoniae and E. coli worldwide is a source of explicit concern. The hospital sewage or the low effectivity of hospital wastewater treatment plants may lead
to the dissemination of ESBL-producing bacteria. The permanent presence of detectable antimicrobial levels within the hospital wastewater treatment may powerfully influence the environment, creating a selective pressure that would be ultimately responsible for the dominance of resistant microorganisms among those present in the habitat.

Our first objective was to generate information on the occurrence of ESBLs producing Enterobacteriaceae (ESBLs-pE) and their antibiotic susceptibility pattern in Wastewater Released from Governmental Hospital of Addis Ababa, Ethiopia. Since currently almost all hospitals in Addis Ababa neither have wastewater treatment plants (WWTPs) nor functional waste stabilization bond. Our second objective was to assess the frequency of the common Enterobacteriaceae and their antimicrobial susceptibility pattern.

**Methods**

**Study setting**

A cross sectional study design was employed from March 1 to April 30, 2021 in Addis Ababa which is the Capital City of the Federal Democratic Republic of Ethiopia, the diplomatic capital of Africa and the seat of different international and regional organizations. The city administration is divided in to ten sub city and 116 woreda. It hosts an estimated 3.2-38 million people, which is a 17% share of Ethiopia's total urban population. Presently, Addis Ababa is experiencing an annual growth rate of 3.8% and is speculated to reach 4.7 million residents by 2030. The city covers a Landmass of 540 square kilo meters. The city is located at the heart of the country, at an altitude ranging from 2,100 meters at Akaki in the south to 3,000 meters at Entoto Hill in the North (21).

Within the city there are 13 government hospitals (five federal, six under Addis Ababa health bureau, one owned by police force and one armed force hospital) distributed throughout 10 sub cities. For this study, it was necessary to pick out major hospitals with a lot of instrumentation, a variety of medical services, higher bed and large run of patients. Hence, hospitals having 200 or more beds and those which provide 10 or more type of medical services are considered as major. Seven governmental hospitals in Addis Ababa meet these criteria. Out of these, 5 hospitals are selected randomly to making a sample size of 38.5%. Ten percent or more samples is considered as a good sample size for small populations (22). The selected hospitals included: Tikur Ambesa Specialized Hospital (TASH) having 543 beds and providing 26 different medical service, St. Paul's Hospital Millennium Medical College (SPHMMC) with 337 beds and providing 25 different medical service, All African leprosy and tuberculosis rehabilitation training center (Alert hospital) with 241 beds and providing 16 different medical service, Yekatit 12 hospital medical college (Y12HMC) with 210 beds and 19 different medical service and Menilik II referral hospital (MIIRH) with 203 beds and 15 different medical service(23).

Laboratory analysis was conducted at Ethiopian public health institute (EPHI) in clinical bacteriology and mycology national referral laboratory in collaboration with food microbiology laboratory. The laboratory has been accredited by Ethiopian National Accreditation Office.

**Sampling Frequency and Sampling Technique**

Hospitals that have higher bed and serving many patients were selected in ten sub- city of Addis Ababa. Based on these five governmental hospitals were included for this study. In each hospital, six sampling sites are employed to collect hospital effluent. These are a manhole used to collect a wastewater originate from adult ward, pediatric ward, labor ward, laboratory unit, laundry unit and a manhole of septic tank (which hold effluent from all source and we termed Mixed). In addition, one sampling unit viz. MDR TB ward was incorporated to collect hospital effluent only in alert hospital.

A total of 100 discrete (that is 50 at the morning and 50 at the afternoon) wastewater samples were collected from each ward/unit wastewater collecting manholes at the sampling site with four hour interval in the study period. This encompassed discrete effluent of 24 from each Y12HMC and MIIRH, 20 from each SPHMMC and ALERT, and 12 discrete effluents from TASH.

A "Grab sampling technique" was applied to collect the most representative samples according to guidelines of wastewater sampling techniques stated on Environment Protection Authority (EPA)(24) and American Public Health Association (APHA)(25). Discrete samples were collected in two rounds in each hospital for two months. The 1st and 2nd round samples were collected within 15 day interval. In each round, the discrete samples were collected two times a day with four hour interval from each sampling sites in each hospital in 150 ml cleaned and sterile microbiological glass bottles. Here 150 ml sterile glass container was used to collect 125 ml wastewater samples.

Hospitals’ effluent Samples were collected during their maximum activity period (usually 10:00 am- 2:30 pm) according to the method used by Nuñez and Morett(10). In addition, samples were collected near the center of flow channel, at approximately 10-15 centimeter of the water depth, where the turbulence was at maximum and the possibility settling was minimized. Grazing (skimming) the water surface or slogging the bottle was avoided. The first sample was collected in the morning 10 -10:30 AM whereas the second sample was collected at 2:20 PM from each sampling site. After taking the sample, the neck of the bottle was wiped with 95% alcohol then the sample bottle labelled with date, code number, and time and sampling site. All samples were collected manually and transported instantly to Ethiopia Public Health Institute (EPHI) food microbiology and clinical laboratories with cold chin (4°C) for bacteriological analysis within six hours of collection. A pair of new, non-powdered, disposable glove, a suitable gown and eye google were used each time while, collecting samples to avoid personal contamination. In the same token, heavy duty glove was used to clean and pick up the cover of manholes at time of sample collection.

**Sampling Site**

In present study, there were different wards and units in the selected hospitals. Each ward/unit generated wastewater having different characteristics. So in order to locate in which sampling site the isolate found, the hospital effluents were collected at different manhole of each units/wards of hospital. The hospital effluents were collected at manhole of the adult ward, pediatric ward, delivery ward, laboratory unit, laundry service unit and at sampling site named
MIXED (for this study purpose only). Here mixed sample indicates a hospital effluent in which its origin holding from different ward/unit and flow together hence it was difficult to identify at which specific unit/ward it came. In addition, wastewater was collected from MDR TB ward in case of Alert hospital only. The sampling site manholes located just at the outlet of wastewater of each ward/unit before discharged in to receiving water/collecting septic tank and at the side of each ward/unit building. The geographical position of the sampling site/unit was obtained and documented.

Data Collection Procedure

The important information's were recorded using a pre-developed data collection form by asking the authorized body (about sampling unit/site, wastewater disinfection and disposal procedures), from record book/file (e.g. number of patients served during study period) and using google map application (for geographical location of the sample). After the sampling unit/site at each hospitals identified and its location as well as the source of wastewater, aseptically with care, the cover of each manholes are lifted up to collect the wastewater then covered immediately. The name of the hospital and sampling unit, time and round of collection, as well as its geographical location are recorded on pre-developed data collection format in addition to, labeling collecting bottle at time of sample collection. All of this information was collected by the principal investigator.

LABORATORY ANALYSIS

Isolation and Characterization of Pure Cultures

For isolation the bacteria, a loopful of a well-mixed sample suspension was inoculated using sterile inoculating loop on to MacConkey agar plate (Oxoid LTD, Basingstoke, Hampshire, England) and incubated aerobically at 37 °C for 18-24 hours.

After incubation for 24 hr. at 37°C, bacterial colonies with distinct coloration and morphology were randomly picked up and sub cultured on to another MacConkey Agar plate for further purification. Then purified colonies with distinct presumptive colonies of each suspected bacterial species and fermentation on MacConkey agar are sub cultured on tryptic soya agar (TSA)/nutrient agar (Oxoid LTD, Basingstoke, Hampshire, England) depending on the availability of media for biochemical test.

For identification pure colony from non-selective nutrient agar/TSA was sub cultured and identified based on the following biochemical tests: oxidase, indole, urea, motility, Lysine decarboxylase, citrate utilization and triple sugar iron tests as per the standards of microbiology procedure including E. coli ATCC 25922, as a control culture (26). Following purification and species identified, two –three purified colonies were preserved in Skimmy milk at -80°C for further characterization, after each isolate was assigned a unique identification number.

Screening isolates for ESBLs Producing

Those Enterobacteriaceae that was resistant or reduced susceptibility to the screening indicator cephalosporin (ceftaxime and/or ceftazidime) was considered as suspicious of ESBLs production. In other word, isolates that showed an inhibition zone size of ≤27 mm for cefotaxime (30 μg) and/or ≤ 22 mm for ceftazidime (30 μg) were considered as suspicious ESBL producers and selected for confirmation for ESBLs production.

Confirmation of ESBLs Producing Enterobacteriaceae

Confirmation of suspicious ESBL-producing isolate was verified by the combination disk method (CDT) as delineated by the 29th edition CLSI guide line (27). The test was performed using two cephalosporin antibiotics: ceftazidime (30 μg), and cefotaxime (30 μg) alone and in combination with beta-lactam inhibitor ((ceftazidime- clavulanic acid (30/10 μg), and cefotaxime-clavulanic acid (30/10 μg)) by dispensing on 0.5 McFarland turbidity bacterial suspension inoculated Muller Hinton agar (MHA) plate (Oxoid LTD, Basingstoke, Hampshire, England) and then incubated overnight (18-24 hours) at 37 °C as per 29th edition CLSI guideline. ESBL production was considered positive when ≥ 5mm increase in the zone diameter for the ceftazidime or cefotaxime tested in combination with clavulanic acid versus its zone when tested alone (27). E. coli ATCC 25922 was used as a negative control throughout the tests as a non-ESBL culture.

Antimicrobial Susceptibility Testing

Once the bacteria were isolated and identified from each sample collected, all Enterobacteriaceae isolates were assessed for non-susceptible pattern for 12 antibiotic agents by using the Kirby-Bauer disk diffusion method on MHA in line with 29th edition CLSI guideline (27). Bacterial inoculum was prepared by suspending the freshly grown bacteria in 4–5 ml sterile normal saline and the turbidity was adjusted to that of a 0.5 McFarland standard. Then a prepared bacterial inoculum suspension (0.5 McFarland standards) was streaked on MHA using sterile applicator stick and antimicrobial discs were placed. The antibiotic discs used for this study were: trimethoprim-sulphamethoxazole (SXT, 1.25/3.75ug), ciprofloxacin (CPR, 5ug), tazobactam+piperacillin (TZP,30ug), cefotixin (CXT, 30ug), chloramphenicol (CHL, 30ug), nitrofurantoin (F, 300 μg), amoxicillin/clavulanic acid (AMC, 20/10 μg), tobramycin (TOP, 10ug), meropenem (MER, 10μg), cefotaxime (CTX, 30ug), cefepime (CFP30 μg), and ceftazidime (CAZ, 30 μg). The antibiotic discs used were the product of Abtek Biologicals Ltd, Liverpool, United Kingdom. Inhibition zones were measured using ruler and isolates were categorized as: resistance, intermediate and susceptible for each antimicrobial agent using the break point as set in line with 29th edition CLSI guidelines (27). The isolates were going to be considered as MDR when they were non-susceptible for three or more classes of antibiotics (28). E. coli ATCC 25922 and P. aeruginosa ATCC 27853 were used for quality control throughout the antimicrobial susceptibility tests as recommended by 29th edition CLSI.

Laboratory Data Quality Assurance

Sample collection, handling, transportation and microbiological analysis and interpretation of results were carried out using standard operating procedures. Before the tangible procedure, reagents, antimicrobial disks, and media were checked for damage, storage problems and expiry date. Laboratory equipment's
are appropriately cleaned and sterilized before use. Media's was prepared according to the respective manufacturer's instruction. Five percent of prepared media per batch was incubated overnight for sterility checkup. Quality control for new batch was performed using ATCC 25922 E. coli standard control to cross check the quality of antibiotics disks and culture media. For ESBLs confirmatory test E. coli ATCC 25922 (ESBLs negative) and K. pneumoniae ATCC 700603 (ESBLs positive) standard control strains are served at time of the procedure [27].

Data analysis and interpretation method

Data was entered and summarized using SPSS version 20 software (IBM Corporation, Armonk, NY, USA). Frequency and percentages of isolates, antibiotic susceptibility pattern of Enterobacteriaceae and ESBLs-pE were calculated. Tables and figures have been employed for data demonstration.

Statistical Data Quality Assurance

Before data entry, data from the data collection form was cross checked for its completeness and accuracy. Culture isolates and antibiotics susceptibility test results had been documented consciously ahead of entry to SPSS. Furthermore, data cleaning and double-data entry was implemented to assure quality of the data.

Results

Distribution of gram-negative bacteria isolates against sampling unit and hospitals

A total of 100 hospital effluent samples were collected and analyzed for the presence of Enterobacteriaceae family. Of these samples 87% were tested positive and contained one or more than one type of isolates. Meanwhile, 183 non-duplicate Gram-negative bacteria were picked from MacConkey agar, 80.3% (147) belonging to Enterobacterial species. The remaining isolates included Pseudomonas spp. (2.2%), Acinetobacter spp. (4.4%), and other unidentified Gram-negative bacteria (13%).

Of 147 Enterobacteriaceae family isolates recovered, the highest distributions were from; laboratory unit 32 (21.8%) and mixed source wastewater 30(20.4%). Whereas the least isolates identified from MDR TB ward effluent (4.8%), which was only collected from ALERT. In this study, the highest number of isolated bacteria, irrespective of total sample collected, were recovered from Y12HMC (34), MIIRH (32) and SPHMMC (32); while, the least isolates were obtained from TASH (21) (Table 1).

Frequency of Enterobacteriaceae Isolates

Among all Enterobacteriaceae the most frequent isolates were E. coli (45.6%), K. pneumoniae (10.2%) and E. cloacae (9.5%) respectively. E. coli was predominantly isolated in laboratory effluent (22.4%) followed by adult ward effluent (19.4%). Meanwhile, K. pneumoniae were mostly obtained from laboratory effluent (26.7%), laundry unit (20%) and mixed source wastewater samples (20%). E. coli and K. pneumoniae were the most frequently isolate identified from pediatric ward (47.4%, 10.5%) and laboratory unit (46.9%, 12.5%) respectively; whereas E. coli and Citrobacter spp. were predominantly isolated from adult ward, (61.9%, 19.5%) and mixed source effluent (33.3%, 13.3%) respectively (Table 1).
### Table 1

Distribution of Enterobacteriaceae isolate against within hospital, sampling unit, time and round of sample collection, at selected governmental hospitals was Ethiopia from April 1 to May 31, 2020.

| Variables (n) | Enterobacteriaceae isolates n(%) |
|---------------|----------------------------------|
|               | E.coli  | K.pneumonia | E.cloacae | Citrobacter spp. | K.ozaene | K.rhinosclro | K.oxytoca | M.morgani | Salmonel |
| **Hospital**   |         |             |           |                |          |              |           |            |          |
| TASH(21)      | 6(28.6) | 2(9.5)      | 4(19)    | 1(4.8)         | 3(14.3)  | 3(14.3)       | 1(4.8)   | 0          | 1(4.8)   |
| SPHMMC(32)    | 17(53.1)| 3(9.4)      | 2(6.3)   | 1(3.1)         | 2(6.3)   | 1(3.1)        | 0         | 1(3.1)    | 1(3.1)   |
| ALERT(28)     | 12(42.9)| 3(10.7)     | 2(7.1)   | 4(14.3)        | 0        | 0             | 2(7.1)   | 2(7.1)    | 1(3.6)   |
| Y12HMC(34)    | 16(47.1)| 3(8.8)      | 3(8.8)   | 4(11.8)        | 1(2.9)   | 3(8.8)        | 2(5.9)   | 1(2.9)    | 0.0      |
| MIIRH(32)     | 16(50)  | 4(12.5)     | 3(9.4)   | 2(6.3)         | 2(6.3)   | 1(3.1)        | 2(6.3)   | 0          | 0        |
| **Sampling Unit** |         |             |           |                |          |              |           |            |          |
| Adult ward(21)| 13(61.9)| 1(4.8)      | 2(9.5)   | 4(19)          | 0        | 0             | 0         | 0          | 1(4.8)   |
| Pediatric ward(19)| 9(47.4)| 2(10.5)    | 2(10.5)  | 1(5.3)         | 2(10.5)  | 1(5.3)        | 1(5.3)   | 1(5.3)    | 0.0      |
| Laboratory unit(32)| 15(46.9)| 4(12.5) | 3(9.4) | 1(3.1) | 1(3.1) | 4(12.5) | 1(3.1) | 1(3.1) | 0.0      |
| Laundry unit(17)| 10(58.8)| 3(17.6) | 2(11.8) | 0 | 0 | 0 | 0 | 0 | 0.0      |
| Labor ward(21)| 7(33.3) | 2(9.5) | 2(9.5) | 1(4.8) | 1(4.8) | 2(9.5) | 3(14.3) | 0 | 1(4.8) |
| Mixed source(30)| 10(33.3)| 3(10) | 3(1) | 4(13.3) | 4(13.3) | 1(3.3) | 2(6.7) | 0 | 1(3.3) |
| MDR TB ward(7) | 3(42.9) | 0 | 0 | 1(14.3) | 0 | 0 | 2(28.6) | 0 | 0.0 |
| **Time of collection** |         |             |           |                |          |              |           |            |          |
| Morning(71)   | 33(46.5)| 10(14.1) | 7(9.9)   | 4(5.6)         | 1(1.4)   | 4(5.6)        | 3(4.2)   | 3(4.2)    | 3(4.2)   |
| Afternoon(76) | 34(44.7)| 5(6.6)    | 7(9.2)   | 8(10.5)        | 7(9.2)   | 4(5.3)        | 4(5.3)   | 1(1.3)    | 0        |
| **Round of collection** |         |             |           |                |          |              |           |            |          |
| First(80)     | 36(45)  | 7(8.8)    | 8(10)    | 6(7.5)         | 5(6.3)   | 5(6.3)        | 5(6.3)   | 2(2.5)    | 2(2.5)   |
| Second(67)    | 31(46.3)| 8(11.9)   | 6(9)     | 6(9)           | 3(4.5)   | 3(4.5)        | 2(3)     | 2(3%)     | 1(1.5)   |
| **Total (N=147)** | 67(45.6)| 15(10.2) | 14(9.5)  | 12(8.2)        | 8(5.4)   | 8(5.4)        | 7(4.8)   | 4(2.7)    | 3(2)     |

Note: =>other isolates were Proteus spp., Shigella spp., Ent. aerogens, Edwardsella, and P. alkalifacience

TASH=Tikur Ambesa Specialized Hospital, SPHMMC=St. Paul’s Hospital Millennium Medical College, ALERT=All African leprosy and tuberculosis rehabilitation training center, Y12HMC=Yekatit 12 hospital medical college, MIIRH=Menilik II referral hospital, MDR TB Ward = multidrug resistant tuberculosis ward.

### Antimicrobial Susceptibility pattern of Enterobacteriaceae

The prevalence of antimicrobial resistant pattern for Enterobacteriaceae isolated ranged from 8.2 to 77.6% in wastewater isolates, with most of the strains susceptible to meropenem (MER) and nitrofurantoin (F). They showed high resistant to amoxicillin-clavulanic acid (77.6%) and trimethoprim/sulfamethoxazole (57.8%). Whereas, meropenem, nitrofurantoin, and tazobactam/piperacillin had the lowest resistant rate 8.2%, 8.2%, 12.9% respectively. In this study the most first, second and third resistant Enterobacteriaceae viz. E. coli, Citrobacter spp. and E. cloacae revealed the highest resistant for amoxicillin-clavulanic acid of 85.1%, 75%, and 85.7% respectively. However, the fourth most resistant K. pneumoniae showed the highest level of resistant against CTX (60%) (Table 2).

Out of 147 Enterobacteriaceae strains tested, 125 (85%) were found resistant to at least one or more antibiotics tested. Hence, 2.7% were resistant to all antibiotics tested (12 drugs), 0.7% resistant to 10 drugs, and 12.9% were resistant to seven drugs (Table 3). In the same talked, 64% (94/147) of tested Enterobacteriaceae were MDR strain. Meanwhile, a total of 62.7% E. coli, 83.3% Citrobacter spp., and 64.3% E. cloacae were identified as the predominant MDR isolates within species (Table 4).
Table 2
Antimicrobial resistant pattern of Enterobacteriaceae identified from different sampling unit at selected governmental hospitals in Addis Ababa, Ethiopia from to May 31, 2020.

| Isolates (number) | Tested Antibiotics Number n (%) |
|-------------------|---------------------------------|
|                   | SX(T) | CPR | TZP | CXT | CHL | F | AMC | TOB | MER | CTX | CFP | CAG |
| E.coli(67)         | 43(64.2) | 34(50.7) | 9(13.4) | 14(20.9) | 4(6) | 0 | 57(85.1) | 18(26.9) | 3(4.5) | 35(52.2) | 32(47.8) | 29(47.5) |
| K.rhinoscler(8)    | 4(50) | 4(50) | 0 | 0 | 2(25) | 3(37.5) | 0 | 7(87.5) | 4(50) | 0 | 4(50) | 4(50) | 4(51) |
| K.pneumonia spp (15) | 7(46.7) | 6(40) | 0 | 0 | 2(13.3) | 0 | 8(53.3) | 5(33.3) | 0 | 9(60) | 8(53.3) | 8(51) |
| Citrobacter spp. (12) | 8(66.7) | 7(58.3) | 1(8.3) | 7(58.3) | 5(41.7) | 2(16.7) | 9(75) | 2(16.7) | 2(16.7) | 6(50) | 5(41.7) | 6(51) |
| Sallmonella spp (3) | 1(33.3) | 2(66.7) | 0 | 0 | 1(33.3) | 0 | 3(100) | 1(33.3) | 1(33.3) | 2(66.7) | 2(66.7) | 2(61) |
| E.cloaceae(14)    | 7(50) | 9(64.3) | 5(35.7) | 9(64.3) | 6(42.9) | 6(42.9) | 12(85.7) | 6(42.9) | 5(35.7) | 8(57.1) | 8(57.1) | 8(51) |
| K.oxytocha(7)     | 2(28.6) | 3(42.9) | 0 | 1(14.3) | 1(14.3) | 0 | 3(42.9) | 2(28.6) | 0 | 3(42.9) | 2(28.6) | 3(41) |
| K.ozanae(8)       | 5(62.5) | 2(25) | 1(12.5) | 4(50) | 2(25) | 0 | 5(62.5) | 3(37.5) | 0 | 5(62.5) | 5(62.5) | 4(51) |
| M.morganii (4)    | 3(75) | 3(75) | 0 | 0 | 0 | 2(50) | 3(75) | 0 | 0 | 3(75) | 3(75) | 3(71) |
| Other isolates (9) | 5(55.6) | 7(77.8) | 3(33.3) | 2(22.2) | 2(22.2) | 2(22.2) | 7(77.8) | 2(22.2) | 1(11.1) | 4(44.4) | 4(44.4) | 4(41) |
| Total non-susceptible (147) | 85(57.8) | 77(52.4) | 19(12.9) | 39(26.5) | 26(17.7) | 12(8.2) | 114(77.6) | 43(29.3) | 12(8.2) | 79(53.7) | 73(49.7) | 71(49) |

Note: SX(T)= Sulfathoxazole-trimethoprim, CRP= Ciprofoxacin, TZP= Tazobactam/Piperacillin, CXT= Cefoxitin, CHL= Chloramphenicol, F= nitrofurantoin, A Amoxicillin-Calvulanic acid, TOB= Tobramycine, MER= Meropenem, CTX= Cefotaxime, CFP=Cefepime, CAZ= Ceftazidime. = other isolates were Proteus spp, Shigella spp, Ent. aerogens, Edwardsella, and P. alkalifaciac.

Table 3
Level of antibiotic resistant of Enterobacteriaceae identified from different sampling units of selected governmental hospitals, Addis Ababa, Ethiopia from Apr to May 31, 2020.

| Isolates (number) | Level of antibiotics resistant ((number (%)) |
|-------------------|------------------------------------------|
|                   | Ro | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 | R12 |
| E.coli(67)         | 6(9) | 8(11.9) | 11(16.4) | 8(11.9) | 4(6) | 7(10.4) | 8(11.9) | 5(7.5) | 4(6) | 4(6) | 1(1.5) | 1(1.5) | 0 |
| K.rhinoscler(8)    | 1(12.5) | 0 | 1(12.5) | 2(25) | 0 | 0 | 1(12.5) | 2(25) | 1(12.5) | 0 | 0 | 0 |
| K.pneumonia(15) | 6(40) | 1(6.7) | 0 | 0 | 0 | 1(6.7) | 2(13.3) | 5(33.3) | 0 | 0 | 0 | 0 |
| Citrobacter spp. (12) | 0 | 0 | 2(16.7) | 2(16.7) | 2(16.7) | 2(16.7) | 1(8.3) | 1(8.3) | 1(8.3) | 0 | 0 | 1(8.3) | 0 |
| Sallmonella spp. (3) | 0 | 1(33.3) | 0 | 0 | 0 | 0 | 0 | 2(66.7) | 0 | 0 | 0 | 0 |
| E.cloaceae(14)    | 2(14.3) | 0 | 3(21.4) | 1(7.1) | 0 | 0 | 1(7.1) | 1(7.1) | 1(7.1) | 0 | 0 | 1(7.1) | 4(28) |
| K.oxytocha(7)     | 4(57.1) | 0 | 0 | 0 | 0 | 1(14.3) | 0 | 1(14.3) | 1(14.3) | 0 | 0 | 0 |
| K.ozanae(8)       | 2(25) | 0 | 1(12.5) | 0 | 0 | 1(12.5) | 2(25) | 0 | 1(12.5) | 1(12.5) | 0 | 0 |
| M.morganii(4)     | 1(25) | 0 | 0 | 0 | 0 | 0 | 0 | 1(25) | 2(50) | 0 | 0 | 0 |
| Other isolates (9) | 0 | 0 | 3(33.3) | 1(11.1) | 1(11.1) | 2(22.2) | 0 | 0 | 0 | 1(11.1) | 0 | 1(11.1) | 0 |
| TOTAL(N=147)      | 22(15) | 10(6.8) | 21(14.3) | 14(9.5) | 7(4.8) | 14(9.5) | 16(10.9) | 19(12.9) | 9(6.1) | 6(4.1) | 1(0.7) | 4(2.7) | 4(2.7) |

Note: Ro: resistant to no antibiotics, R1-12: resistant to 1, 2, 3, 4, 5, 6-12 antibiotics used at time of study. = other isolates were Proteus spp, Shigella spp, Ent. aerogens, Edwardsella, and P. alkalifaciac.
Occurrence of MDR and ESBLs producing Enterobacteriaceae spp. within hospital and sampling units as well as within MDR and ESBLs producer at selected hospitals, in Addis Ababa, Ethiopia from April 1 to May 31, 2020.

| Hospitals isolates n, Vs ESBLs positive and MDR yes n(%) | Sampling unit n(%) | Wastewater collected time |
|---------------------------------------------------------|-------------------|--------------------------|
|                                                         | Adult ward        | Pediatric ward           | Laboratory | Laudnary | Labor ward | Mixed source | MDR TB ward | Morning | Afternoon |
| TASH,21 ESBLs positive 11(52.4)                           | 1(9.1)            | 2(18.2)                  | 2(18.2)    | 1(9.1)   | 2(18.2)    | 3(27.3)      | NC         | 4(36.4) | 7(63.6)   |
| MDR yes 16(31.2)                                         | 1(6.3)            | 4(25)                    | 4(25)      | 1(6.3)   | 3(18.8)    | 3(18.8)      | NC         | 8(50)   | 8(50)     |
| SPHMMC,32 ESBLs positive 15(46.9)                         | 2(13.3)           | 3(20)                    | 2(13.3)    | 4(26.7)  | NC         | 4(26.7)      | NC         | 7(46.7) | 8(53.3)   |
| MDR yes 22(68.8)                                         | 4(18.2)           | 3(13.6)                  | 4(18.2)    | 5(22.7)  | NC         | 6(27.3)      | NC         | 11(50)  | 11(50)    |
| ALERT,28 ESBLs positive 19(67.9)                          | 4(21.1)           | NC                       | 6(31.6)    | 1(5.3)   | 4(21.1)    | NC           | 4(21.1)    | 9(47.7) | 10(52.6)  |
| MDR yes 20(71.4)                                         | 3(15)             | NC                       | 6(30)      | 1(5)     | 4(20)      | NC           | 6(30)      | 10(50)  | 10(50)    |
| Y12HMC,34 ESBLs positive 16(47.1)                         | 4(25)             | 2(12.5)                  | 1(6.3)     | 0        | 3(18.8)    | 6(37.5)      | NC         | 8(50)   | 8(50)     |
| MDR yes 19(55.9)                                         | 4(21.1)           | 3(15.8)                  | 1(5.3)     | 0        | 4(21.1)    | 7(36.8)      | NC         | 8(42.1) | 11(57.9)  |
| MIIRH,32 ESBLs positive 10(51.2)                          | 3(30)             | 0                        | 2(20)      | 4(40)    | 1(10)      | 0            | NC         | 6(60)   | 4(40)     |
| MDR yes 17(53.1)                                         | 3(17.6)           | 0                        | 5(29.4)    | 5(29.4)  | 2(11.8)    | 2(11.8)      | NC         | 8(47.1) | 9(52.9)   |
| Total, N=147 ESBLs positive N=71(48.3%)                    | 14(19.7)          | 7(9.9)                   | 13(18.3)   | 10(14.1) | 10(14.1)   | 13(18.3)     | 4(5.6)     | 34(47.9)| 37(52.1)  |
| MDR yes N=94(64%)                                        | 15(16)            | 10(10.6)                 | 20(21.3)   | 12(12.8) | 13(13.8)   | 18(19.1)     | 6(6.4)     | 45(47.9)| 49(52.1)  |

Note: TASH=Tikur Ambesa Specialized Hospital, SPHMMC= St. Paul’s Hospital Millennium Medical College, ALERT= All African leprosy and tuberculosis rehabilitation training center, Y12HMC= Yekatit 12 hospital medical college, MIIRH= Menilik II referral hospital, MDR TB Ward = multidrug resistant tuberculosis ward, NC= sample not collected.

Magnitude of ESBLs Producing Enterobacteriaceae

Of all Enterobacteriaceae 55.1% (81/147) were suspected as potential ESBLs producing with screening method of cefotaxime zone of inhibition ≤ 27 mm and ceftazidime zone of inhibition ≤ 22 mm (Figure 1). Out of 81 ESBLs potential Enterobacteriaceae, 71 (87.7%) were found to be ESBLs producing isolates by the combination disk test. The overall magnitude of ESBLs producing Enterobacteriaceae (ESBLs-pE) were 48.3% (71/147,) with the highest percentage found in *E. coli* (21.8%), *K. pneumoniae* (4.8%) and *Citobacter* spp. (4.8%); while, the lowest ratio observed in *Salmonella* spp. 1.4% (2/147) respectively (Figure 2).

The occurrence of ESBLs producers differ strongly within different species of Enterobacteriaceae. The highest within-species frequency of ESBLs production was recovered among *M. morganii* (75%) pursued by *Salmonella* spp. (66.7%) and *K. ozanae* (62.5%) respectively. However, least within-species ESBLs production was found in *E. cloaca* (21%) (Table 5 and Figure 2).
The magnitude of ESBLs producing and MDR Enterobacteriaceae in the wastewater were different in the five hospitals. The highest occurrence of ESBLs-pE within hospital according to CDT identification method was found in wastewater from the ALERT (67.9%), followed by TASH (52.4%) and Y12HMC (47.1%) respectively. Whereas the least ESBLs-pE within hospital occurred in MIIRH (31.2%) (Table 5). In contrary, the elevated MDR isolates within hospital were observed in wastewater of TASH (76.2%) and ALERT (71.4%); while, the lowest ratio was found in the same way as ESBL producer isolates in MIIRH (53.1%) (Table 6).

Note: TASH=Tikur Ambesa Specialized Hospital, SPHMMC= St. Paul’s Hospital Millennium Medical College, ALERT= All African leprosy and tuberculosis referral center, Y12HMC= Yekatit 12 hospital medical college, MIIRH= Menilik II referral hospital, MDR TB Ward = multidrug resistant tuberculosis ward.
The magnitude of MDR and ESBLs-pE obtained from all wastewater samples were higher at the afternoon than the morning wastewater collected. At morning effluent the occurrence of MDR isolates and ESBL producers within time of wastewater collection were 59.2% and 47.9%, whereas at the afternoon were 64.5% and 52.1% respectively. On the contrary, they were higher in the first round of effluent collection than the second. In the first round MDR isolates and ESBL producers were 77.6% and 48.8%, while in the second round effluent collection they were 62.7% and 40% correspondingly (Table 5 and Table 6).

Table 6
Prevalence of MDR Enterobacteriaceae species within hospital, sampling unit, time and round of wastewater collection as well as within species and MDR isolates at selected governmental hospitals, in Addis Ababa, Ethiopia from April 1 to May 31, 2020.

| MDR YES, n (%) | Enterobacteriaceae isolates recoverd from hospital effluents n(%) |
|---------------|-------------------------------------------------------------|
|               | E. coli | Citrobacter spp. | E. cloacae | K. pneumonia | K. rhinoscler | K. ozanae | K. oxytoca | M. morganii | Salmonellae pp. | Other isolate |
| wastewater collected Hospitals |         |                  |           |              |              |          |            |              |                 |               |
| TASH, 16(76.2) | 3(18.8) | 1(6.3)           | 4(25)     | 2(12.5)      | 2(12.5)      | 1(6.3)   | 0          | 1(6.3)      | 0               |
| SPHMMC, 22(68.8) | 10(45.5) | 1(4.5)          | 1(4.5)    | 2(9.1)       | 1(4.5)       | 2(9.1)   | 0          | 1(4.5)      | 1(4.5)          | 3(13.6)        |
| ALERT, 20(71.4) | 9(45)   | 3(15)           | 1(5)      | 2(10)        | 0            | 0        | 2(10)      | 2(10)       | 0               | 1(5)           |
| Y12HMC, 19(55.9) | 9(47.4) | 4(21.1)         | 1(5.3)    | 1(5.3)       | 2(10.5)      | 1(5.3)   | 0          | 0           | 0               | 1(5.3)         |
| MIIRH, 17(53.1) | 11(64.7) | 1(5.9)         | 2(11.8)   | 1(5.9)       | 1(5.9)       | 0        | 0          | 0           | 0               | 1(5.9)         |
| wastewater sampling unit |         |                  |           |              |              |          |            |              |                 |               |
| Adult ward, 15(71.4) | 9(60)   | 3(20)           | 2(13.3)   | 1(6.7)       | 0            | 0        | 0          | 0           | 0               | 0              |
| Pediatric ward,10(52.5) | 5(50)   | 1(10)           | 1(10)     | 1(10)        | 0            | 2(20)    | 0          | 0           | 0               | 0              |
| Laboratory, 20(62.5) | 7(35)   | 1(5)            | 2(10)     | 2(10)        | 3(15)        | 1(5)     | 1(5)       | 1(5)        | 0               | 2(10)          |
| Laundry, 12(70.5) | 8(66.7) | 0               | 1(8.3)    | 2(16.7)      | 0            | 0        | 0          | 0           | 0               | 1(8.3)         |
| Labor ward, 13(61.9) | 4(30.8) | 1(7.7)          | 2(15.4)   | 0            | 2(15.4)      | 1(7.7)   | 1(7.7)    | 0           | 1(7.7)          | 1(7.7)         |
| wastewater collected Time |         |                  |           |              |              |          |            |              |                 |               |
| Morning, 45(63.4) | 18(40)  | 3(6.7)          | 5(11.1)   | 6(13.3)      | 4(8.9)       | 1(2.2)   | 1(2.2)    | 2(4.4)      | 2(4.4)          | 3(6.7)         |
| Afternoon, 49(64.5) | 24(49)  | 7(14.3)         | 4(8.2)    | 2(4.1)       | 2(4.1)       | 4(8.2)   | 2(4.1)    | 1(2)        | 0               | 3(6.1)         |
| wastewater collected round |         |                  |           |              |              |          |            |              |                 |               |
| First, 52(65) | 22(42.3) | 5(9.6)         | 5(9.6)    | 4(7.7)       | 3(5.8)       | 3(5.8)   | 3(5.8)    | 2(3.8)      | 2(3.8)          | 3(5.8)         |
| Second, 42(62.7) | 20(47.6) | 5(11.9)        | 4(9.5)    | 4(9.5)       | 3(7.1)       | 2(4.8)   | 0         | 1(2.4)     | 0               | 3(7.1)         |
| TOTAL          |         |                  |           |              |              |          |            |              |                 |               |
| Total MDR N=94 | 42(44.7) | 10(10.6)        | 9(9.6)    | 8(8.5)       | 5(6.4)       | 5(5.3)   | 3(3.2)    | 3(3.2)      | 2(2.5)          | 6(6.4)         |
| Total Isolate N=147 | 67(62.7) | 12(83.3)        | 14(53.3)  | 8(62.5)      | 8(62.5)      | 7(42.9) | 4(75)     | 3(66.7)     | 9(66.7)         |               |

Note: TASH=Tikur Ambesa Specialized Hospital, SPHMMC= St. Paul’s Hospital Millennium Medical College, ALERT= All African leprosy and tuberculosis rehabilitation training center, Y12HMC= Yekatit 12 hospital medical college, MIIRH= Menilik II referral hospital, MDR TB Ward = multidrug resistant tuberculosis ward.

Antibiotics Susceptibility pattern of ESBLs producing Enterobacteriaceae to potentially active drugs

The antibiotic resistant profile for ESBLs producing and non ESBLs producer Enterobacteriaceae are displayed in Figure 3 below. The antibiotics susceptibility pattern of ESBLs-pE were also performed in relation to potential active antibiotics like carbapenems (meropenem), quinolone/fluroquinolone (ciprofoxacin), cephemycine (cefoxitin) and amino glycoside (tobramycin) drug Categories. In addition, it was tested against for combinantion drug (amoxaciline-clavunlate and peparcillin-tazobactam), phenicol (chloramphenicol), nitrofuran (nitrofurantoin) and folate pathway antagonist (trimthoprime-sulfamethoxazole) drug families.
The predominant ESBLs-pE was found to be more than 85% resistant, the highest resistant levels were observed on cefotaxime (95.8%), amoxicillin/clavulenate (93%) and cefepime (90.1%). The most common co-resistance rates among the ESBLs-pE isolates to ciprofloxacin and trimthoprim-sulfamethoxazole were 74.6% and 73.2% respectively. Meanwhile, aminoglycoside and cephamycin such as tobramycin (40.8%) and cefoxitin (29.6%) showed reduced efficacy against the ESBLs-pE. However, the most active drugs for ESBLs producing isolates were meropenem, nitrofurantoin and piperacillin/tazobactam with susceptibility 94.4%, 88.7% and 87.3% correspondingly. Out of confirmed ESBLs-pE the highest ESBLs production was observed among E. coli with 45% (32/71) prevalence. While, the least ESBLs production from the total ESBLs positive Enterobacteraeae was detected in salmonella spp. with 2.2% (2/71) ratio (Table 5).

Non-ESBLs producers Enterobacteriaceae showed resistant level of 63.2%, 43.4% and 31.6% to amoxicillin/clavunulate, trimthoprime-sulfamethoxazole and ciprofloxacin respectively. In addition, nitrofurantoin, chloramphenicol and meropenem showed least resistant with 7.9%, 10.5 and 10.5% respectively.

**Discussion**

The occurrence of antibiotic resistant and ESBLs-pE from hospital wastewater could be exceptionally problematic because of the ability of nosocomial pathogens to transfer antibiotic resistance genes among different hosts and environments. The dissemination of MDR bacteria via hospital wastewater is a usuable cause for concern, because it is reasonable that MDR bacteria are selected mostly in hospitals and take away by wastewater. This study, explains about the prevalence, the occurrence of MDR and ESBLs-pE from the five selected governmental hospitals wastewater in Addis Ababa, Ethiopia.

**Prevalence of Enterobacteriaeae isolates**

In the present study the most frequent Enterobacteriaceae isolates were E. coli (45.6%), K. pneumoniae (10.2%) and E. cloacae (9.5%). Our findings were comparable figure to other studies, in Addis Ababa; E. coli (32%), K. pneumonia (15%) and E. cloacae (6%) (31), in South Eastern, Nigeria; E. coli (26.2%) (32), in Luzhou City in Sichuan province, China; E. coli (56.5%), K. pneumoniae (27.4%) and Enterobacter spp. (8.1%) (33), in Bangladesh; E. coli and Klebsiella spp. (30.7% each) and Enterobacter spp. (25%) (34).

However, our finding was a little dissimilar to other studies conducted, in Northwest Ethiopia; from hospital environment Klebsiella spp. (29.2%), E. coli (12.3%) and Enterobacter spp. (3.1%) (35), in Mekelle: from untreated hospital wastewater Klebsiella spp. (25.9%), and E. coli (21.2%) (36), in Biratnagar, Nepal; from effluents of different hospitals sewage E. coli (34.7%), Citrobacter (21.7%), Enterobacter (21.7%), and Klebsiella (13%) (37). These variations might be due to sample type (inanimate object and swage of hospital), study period, sample size and type of pathogen infecting patients at time of sample collection.

**Antibiotics resistant pattern of Enterobacteriaceae isolates**

In the present study, the overall prevalence of antimicrobial resistant pattern for Enterobacteriaceae isolated ranged from 8.2 to 77.6% in wastewater isolates, with most of the strains susceptible to meropenem (MER) and nitrofurantone (F). This finding was in line with study conducted in Rio de Janeiro, Brazil, with 0 to 83% resistant range for Gram negative isolates and most strains susceptible to meropenem (38).

In this study, out of 147 Enterobacteriaceae strains tested, 125 (85%) were found resistant to at least one or more antibiotics tested. Meanwhile, Enterobacteriaceae strains showed the highest resistant to amoxicillin-clavulanic acid (77.6%) followed by trimethoprim/sulfamethoxazole (57.8%), cefotaxime (53.7%), and ciprofloxacin (52.4%).

This finding more or less correlates with other study conducted in China; trimethoprim/sulfamethoxazole (77.4%), amoxicillin-clavulanic acid (66.1%), and ciprofloxacin (61.3%) (33) had relatively higher resistance. However, our finding disagrees with previous study conducted where lower resistant proportion reported, in Northwest Ethiopia (Gondar): trimethoprim/sulfamethoxazole (29.8%), cefotaxime (23.8%), ciprofloxacin (10.6%) (35), and in Bangladesh; ciprofloxacin (23%) (34).

A study done in China, showed high resistance of Enterobacteriaceae for cefotaxime (100%), meropenem (51.6%) and chloramphenicol (48.4%) (33), contradicting the results presented herein where less resistant observed for cefotaxime (53.7%), chloramphenicol (17.7%) and meropenem (8.2%), again in Rio de Janeiro, Brazil: from the influent wastewater Gram negative isolates showed resistant against cefotaxime (44%), trimethoprim/sulfamethoxazole (34%), ciprofloxacin (17%), and meropenem (3%) (38), which were slightly deviate from current study except meropenem.

In present study, MDR strains were mostly observed in the tested Enterobacteriaceae isolates by 64%. Almost similar MDR isolate results with ours were recorded in study carried out, in Northwest Ethiopia (Gondar) 81.5% (from hospital environment) (35), in Mekelle: 61.5% (from untreated hospital wastewater) (36), in Biratnagar, Nepal; 69.6% (37), in China: 85.5% (33). However, our report contradicted by the previous study conducted in South Eastern, Nigeria (from three hospital effluents) where all the Enterobacteriaceae isolates recovered (E. coli and Salmonella spp.) were MDR although their patterns of resistance varied (32). In the same talked in this study, a total of 83.3% Citrobacter spp., 64.3% E. cloacae, 62.7% E. coli, and 53.3% K. pneumoniae isolates were identified as the predominant MDR. Our finding, was concordant with other previous studies where the common MDR isolates were, in Addis Ababa; Citrobacter (100%), E. cloacae (66.7%) and E. coli (28.6%) (31), in Biratnagar, Nepal; Enterobacter spp. (100%), Citrobacter spp. (80%), E. coli (62.5%), Klebsiella spp. (33.3%) (37). However, our finding dissimilar with a study carried out in China (E. coli (91.4%) and K. pneumoniae (94.1%)) (33), Ibadan, Nigeria (E. coli (94.8%)) (39), and in Biratnagar, Nepal (Enterobacter spp. (100%)) (37), where the highest MDR proportion for E. coli, K. pneumonia and Enterobacter spp. were indicated.

**Magnitude of Extended Spectrum B-lactamase producing Enterobacteriaceae**
In the present study, all Enterobacteriaceae 55.1% were suspected as potential ESBLs producing and 87.7% of them were confirmed ESBLs producing isolates. A little comparable result was reported in Dubai, UAE by Khan MA. et al 2020: among all isolates from municipality wastewater 57.4% suspicious and 25.7% confirmed ESBLs producer Enterobacteriaceae were reported (40), in Northern Italy: 45.4% beta-lactamases producing Enterobacteriaceae were recovered from WWTPs (41). The difference with our result might be due to the type of sample used (hospital Vs municipality wastewater), method used to confirm potential ESBLs producer (CDT Vs DDST) and sample size.

According to the present study, the overall magnitude of ESBLs-pE were 48.3% which is almost in line with a study conducted in Rio de Janeiro, Brazil (38), and Nepal (37) with ESBLs producer isolates of 39%, and 30.4% respectively. In contrast to the current study, other studies conducted in Ethiopia and other countries reported lower prevalence of ESBLs-pE, in Addis Ababa: 25% from hospital wastewater (31), in Northwest, Ethiopia: 14.8% from hospital environment (42), and in Austria: 27.4% from activated sludge (43), were recovered (43). The difference in the prevalence of ESBLs producer in different studies from wastewater isolates might be due to difference in geographic areas, source of sample, period of study (ESBL rapidly changing over time), sample size, method of ESBL detection and an infection control system.

In the current study, the highest percentage of ESBLs-pE was detected in E. coli (45.1%), K. pneumoniae (9.9%), and Citrobacter spp. (9.9%) that is comparable results to study conducted in, Austria with E. coli (65.6%), and K. pneumoniae (22.6%) (43) and in Ibadan, Nigeria E. coli (29.3%) (39). However, the highest ESBLs producer prevalence was documented in K. pneumoniae other than E. coli or other Enterobacteriaceae spp. in other studies which contradict with the present study. Hence, the predominate ESBLs-pE isolate in other studies were, in Addis Ababa; Citrobacter spp. (33.3%), K pneumonia (33.3%), and E. coli (20%) (31), in Northwest, Ethiopia, K. pneumoniae (42.10%), and E. coli (35.09%) (42), in Rio de Janeiro, Brazil: K. pneumonia (41.5%), and E. coli (12.2%) (38), and in Nepal; Enterobacter spp. (60%), Citrobacter spp. (40%) and E. coli (25%) (37). This variation is occurred because of the difference of, wastewater type (hospital Vs municipality), source of wastewater contaminant, the prevalence of microbes, geographical location and disease epidemiology.

The occurrence of ESBLs producers differ strongly within different species of Enterobacteriaceae. The highest within-species frequency of ESBLs production was recovered among M. morganii (75%) pursued by Salmonella spp. (66.7%) and K. ozanae (62.5%) respectively. Meanwhile, least within-species ESBLs production was found in E. cloaca (21%). The difference of the occurrence of ESBLs producer between within ESBLs producer and within species might be the variation of, the number of isolate recovered from the sample, and the isolate compared with (that is comparison within among ESBLs producer Enterobacteriaceae Vs within among each species).

**Distribution of MDR and ESBLs Producing Enterobacteriaceae against the independent variables**

In the present study, of all MDR Enterobacteriaceae, 73.4% were ESBLs producer, whereas only 26.6% of them were non-ESBLs producer Enterobacteriaceae. The magnitude of ESBLs producing and MDR Enterobacteriaceae in the wastewater were different in the five hospitals. The highest occurrence of ESBLs-pE within hospital according to CDT identification method were found in wastewater from the ALERT (67.9%), followed by TASH (52.4%) and Y12HM (47.1%) respectively, whereas; the least ESBLs-pE were detected in MIIRH (31.2%). In contrary, the elevated MDR isolates within hospital were observed in wastewater of TASH (76.2%) and ALERT (71.4%), while the lowest ratio was found in the same way as ESBL producer isolates in MIIRH (53.1%). It is difficult to compare directly the occurrence of MDR and ESBLs-pE in hospital effluent from one country to other because of the presence of difference in geographical zone, the epidemiology of disease (the severity and disease type), the number of patients served, the service provided in the hospital and wastewater disposal police from country to country. However, like our country there were different ESBLs producer occurrence within country hospital effluent; in Ibadan, Nigeria; more ESBLs producer was found ina privately-owned hospital (33.3%) than a State Government-owned hospital (29.1%) (39), in Europe: the elevated ESBLs-pE was found in effluents from the Slovenian general hospital, followed by the Austrian private rehabilitation clinic and the Austrian private surgery clinic (44).

From all hospital wastewater collected for this study purpose, the highest ratio of ESBLs-pE within sampling unit was observed in adult ward effluent (66.7%) followed by laundry unit (58.8%) and labor ward effluents (47.6%) respectively; whereas, the least proportion was recovered in pediatric ward (36.9%) and laboratory unit effluent (40.6%). Similarly with less difference; the elevated MDR isolates within sampling unit were identified in adult ward effluent (71.4%) pursued by laundry unit (70.6%) and laboratory unit effluents (62.5%) correspondingly, while; the lowest ratio was found in pediatric ward effluent (52.6%). In MDR TB ward wastewater which was collected only in ALERT hospital, the proportions of ESBLs-producing and MDR Enterobacteriaceae within sampling unit were 57.1% and 85.7% respectively. Almost all the preceding publication on antibiotic resistant profile of pathogenic microbes has been focused towards crude hospital wastewater rather than at each refined source of it. As a result, it was difficult to compare our result directly with other studies conducted from hospital wastewater, anyway a study conducted in hospital environment in Gondar, reported from inanimate object of medical ward, surgical ward and Gyn-obs ward ESBLs-pE of 52.6%, 10.5% and 5.3% respectively (42). The variation in ESBLs-producing and MDR Enterobacteriaceae proportion within sampling unit in the present study might probably be attributed to the difference in type of patients served, length of patient stay, type of medical service provided, infection prevention and control procedure in each department/unit.

In this study, the magnitude of MDR and ESBLs-pE obtained from all wastewater samples were higher at the afternoon than the morning wastewater collected. At the afternoon effluent, the occurrence of MDR and ESBL producer isolates within time of wastewater collection were 64.5% and 52.1%, whereas; at the morning effluent, were 59.2% and 47.9% respectively. This difference most probable happen because of the majority medical activity performed around the afternoon and outpatients number increase at the afternoon due to transportation and other reasons. On the other hand, MDR and ESBLs-pE were higher in the first round of effluent collection than the second. In the first round, they were 77.6% and 48.8%, while in the second round effluent collection, they were 62.7% and 40% correspondingly.

**Antibiotics susceptibility pattern of ESBLs producing Enterobacteriaceae**

In current study, the predominant ESBLs-pE were found to be more than 85% resistant to the antibiotic like cefotaxime (95.8%), AMC (93%), cefepime (90.1%), and ceftazidime (87.3%). These were in close agreement with other study done in, Northwest Ethiopia; amoxicillin/clavulanic acid (100%) and ceftazidime
(100%), Dubai, UAE; from municipality wastewater, cefotaxime (86%) and ceftazidime (77%) (40), Austria; amoxicillin/clavulanic acid (53.1%) (43) had higher resistance for ESBLs-pE.

In this study, the most common higher co-resistant rates among the ESBLs-pE isolates were 74.6% for ciprofloxacin and 73.2% for SXT. Whereas, aminoglycoside and cephamycine such as tobramycine (40.8%) and cefoxitine (29.6%) showed reduced efficacy against the ESBLs-pE. Our result was comparable with other studies taken place, in Northwest Ethiopia; ciprofloxacin (43.9%), and trimethoprim/sulfamethoxazole (SXT) (64.9%) (42), in Austria; ciprofloxacin (56.3%), trimethoprim/sulfamethoxazole (50%) and cefoxitin (25%) (43).

In our work, nearly the most active drugs for ESBLs producing isolates observed are meropenem, nitrofurantoin, and piperacillin/tazobactam with susceptibility 94.4%, 88.7%, and 87.3% and 74.6% respectively. The findings of this study nearly concordance with prior reports conducted in Austria; meropenem (100%), and piperacillin/tazobactam (90.6%) had good susceptibility level (43).

Non-ESBLs producers Enterobacteriaceae were 63.2%, 43.4%, 31.6% and 23.7% resistant to amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole, ciprofloxacin and Cefoxitin respectively. However, nitrofurantoin, chloramphenicol and meropenem showed least resistant with 7.9%, 10.5 and 10.5% respectively. We observed in the present study ESBLs producer isolates were more resistant to the tested antibiotics than non-ESBLs producer Enterobacteriaceae. This difference might be from the resistant gene on ESBLs producer also contributed the isolate to develop resistance to other antibiotic too.

**Strength of the study**

Our study tried to collect the wastewater, at their utmost source, which enables to take preventive measure and at large sample size (in relative to previous study) to be representative. This study conducted at different governmental higher hospitals to display the extent of distribution of MDR and ESBLs-pE in each hospital sampling units/sites.

**Limitation of the study**

In this study only wastewater was used, hence it was unable to differentiate the source of resistant bacteria either it was from clinical isolates or sewage system.

Our study was unable to select all antimicrobial agents commonly used for resistance evaluation due to the fact that some of them were not available during the period of study.

The major limitation of the study was that ESBL detection was only performed phenotypically using CDT method, it was better to include genotypic method of detection.

Some source of hospital wastewaters were not incorporated in the study. So, to generalize the distribution of MDR and ESBLs-pE in hospital wastewater, it was better assessing all source of wastewater in selected hospitals.

It would have a better figurative data if the study was also include private hospitals wastewater found in Addis Ababa and WWTP of the city.

**Conclusion**

The hospital wastewater released directly into urban sewerage systems without appropriate disinfection or treatment is the serious environmental attention of the day. In this study, there was high magnitude of MDR & ESBLs-pE (≈65% Vs 50%) from the wastewater of selected governmental hospitals in Addis Ababa, Ethiopia. Majority of them were in adult ward and laundry unit effluents. In addition, the most frequent ESBLs-pE were *E. coli, K. pneumoniae* and *citrobacter* spp. In addition, ESBLs-pE showed high rate of resistance to all tested antibiotics as compared to non-ESBLs-E. This is a warning threat to such infection via contamination of food and water from rivers and urbane drainage which will be polluted through untreated hospital wastewater. Hence, infection prevention and control implementation at each hospital is mandatory.

One of the persistence concerns due to the existence of ESBL-producing bacteria in water bodies are related with the transmission of conjugative plasmids, which additionally carry genes of resistant to sulfonamides and aminoglycosides, giving the bacteria multi-resistant patterns (45). This phenomenon was observed in our study, where the most common higher co-resistant rates among the ESBLs-pE identified for ciprofloxacin (74.6%) and SXT (73.2%). However, in this study, the most active drugs for ESBLs-pE were meropenem (94.4%), nitrofurantoin (88.7%) and piperacillin/tazobactam (87.3%).

During sample collection in this study we noticed the selected hospitals had neither WWTP nor wastewater stabilization pond except in TASH nonfunctional waste stabilization pond was observed. Since the present study detected a high proportion of pathogenic, resistant and ESBLs-pE, indicating a higher probability potential risk of microbial pollution of water bodies in the community, hence accelerate spreading of resistant microorganisms into community.

**Recommendation**

The high occurrence of MDR and ESBLs-pE in the present study from hospital effluent may have a severe consequence in the public health. These bacteria can carry several genetic determinants which can be transmitted to another bacterium including pathogens. This indicates a need for a highly committed and consistently hygienic treatment of effluent (implementation of final disinfection procedure to minimize the microbial burden) at each respective wards and units to inhibit the transmission of the antibiotic resistant to another enteric pathogenic bacterium. Since all selected hospitals for this study purpose were hadn't any either wastewater treatment plant or functional wastewater stabilization pond, therefore every concern bodies should take urgent measures to
minimize the devastating outcome from the discharge of hospital wastewaters into the community drainages without getting appropriate treatment. We suggest additional studies to take place on molecular epidemiology of ESBLs producing Enterobacteriaceae and their effects on patient recovery and health care burden in Addis Ababa, Ethiopia.

Abbreviations

ALERT
All African leprosy and tuberculosis rehabilitation training center
AMR
Anti-Microbial Resistance
ATCC
American Type Culture Collection
CDT
combination disk Test method
CFU
Colony Forming Unit
CLSI
Clinical and Laboratory Standard Institute
CTX-M
Cefotaximases Munichin
DDST
Double Disk synergy Test
ESBL
Extended Spectrum Beta- Lactamase
ESBLs-pE
ESBLs producing Enterobacteriaceae
ICU
Intensive Care Unit
MDR
Multi-drug Resistance
MDR TB Ward
Multidrug resistant tuberculosis ward
MHA
Mueller Hinton Agar
MIIRH
Menilik II referral hospital
SHV
SulfHydryl Variable
SPHHMMC
St. Paul's Hospital Millennium Medical College
TASH
Tikur Ambesa Specialized Hospital
TEM
Temoneira
UTIs
Urinary Tract Infections
WWTP
Wastewater Treatment Plant
Y12HMC
Yekatit 12 hospital medical college.

Declarations

Ethics approval
The study proposal was reviewed and verified by the department of research and ethics review committee of the medical laboratory sciences, College of Health Sciences; Addis Ababa University (Ref. No. MLS/223/17). Approval and permission again obtained from the respective hospital in which the isolate and data were collected by their authorized bodies.

Consent for publication
Not applicable
Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

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Antibiotics disks and media were supplied by EPHI. However, the other costs were covered by the principal investigator. The supplier body had no influence on study design, data collection, analysis and interpretation of data and writing the manuscript.

Authors' contributions

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

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Figures
Figure 1

The percentage of ESBLs positive, ESBLs negative and non-potential ESBLs Enterobacteriaceae at selected governmental hospitals wastewater, Addis Ababa, Ethiopia April 1 to May 31, 2020.

Figure 2

Frequency of total, potential and confirmed ESBLs producing Enterobacteriaceae species from different sampling units at selected governmental hospitals wastewater, in Addis Ababa, Ethiopia from April 1 to May 31, 2020.
Figure 3

Antibiotics Non-susceptible pattern of ESBLs positive and Non-ESBLs Enterobacteriaceae to different classes of antibiotics at selected governmental hospitals, Addis Ababa, Ethiopia April 1 to May 31, 2020. Note: SXT= Sulfamethoxazole-trimethoprim, CRP= Ciprofloxacin, TZP= Tazobactam/Piperacillin, CXT= Cefoxitin, CHL= Chloramphenicol, F= nitrofurantoin, AMC= Amoxicillin-Clavulanic acid, TOB= Tobramycin, MER= Meropenem, CTX= Cefotaxime, CFP= Cefepime, CAZ= Ceftazidime.