

## Research Article

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# Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta Lactamase Producers in Gram-negative Urine Isolates at MBN Clinical Laboratories, Kampala Uganda

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## Abstract

**Introduction:** Occurrence of Extended Spectrum beta-lactamase (ESBLs) producing bacteria have presented impediment in treatment choices for urinary tract infections. ESBLs embody a major cluster of lactamases accountable for resistance to novel generations of  $\beta$ -lactam drugs worldwide. The study determined prevalence of ESBL organisms in urine isolates and susceptibility patterns to 13 antibacterial agents.

**Materials and methods:** Two hundred samples were cultured on blood agar, MacConkey agar and incubated at 37°C utmost 48 hours. Isolates identified based on standard bacteriological culture and biochemical characteristics. Drug susceptibility centered on Clinical Laboratory Standard Institute recommended and WHO modified Kirby-Bauer disc diffusion methods. Isolates with reduced susceptibility to Ceftazidime were considered to be possible ESBL producers. Phenotypically confirmed ESBL required use of Ceftazidime in combination with Clavulanic acid. A five millimeter increase zone diameter for Ceftazidime in combination with Clavulanic acid versus its zone tested alone was considered as ESBL.

**Results:** Out of 200 samples, 45 (22.5%) had significant growth, majority *Escherichia coli* 28 (62.2%), *Klebsiella pneumoniae* 11 (24.4%) followed and *Citrobacter freundii* 2 (4.4%). *Enterobacter species*, *Morganella morganii*, *Proteus mirabilis* and *Serratia marcescens* each 1 (2.2%). Prevalence of ESBLs was 56%. Out of 25 (56%) ESBLs, highest prevalence was among *Escherichia coli* (15/25; 60%) followed by *Klebsiella pneumoniae* (5/25; 24%) while *Citrobacter freundii*, *Enterobacter species*, *Morganella morganii* and *Proteus mirabilis* each had (1/25; 4%). Susceptibility was highest to Imipenem 22 (88%) and least to Ampicillin, Amoxicillin-Clavulanic acid and Tetracycline each 1 (4%). No susceptibility to Cefuroxime and Ceftazidime was observed.

**Conclusion:** The study showed that resistant ESBLs generating bacteria were present among urine isolates. We recommend that ESBLs bacteria isolated in urine be treated based on antibiotics susceptibility, continuous surveillance to guide correct treatment for urinary tract infection and to prevent the occurrence of multi drug resistant bacteria. This should be reflected in the policies developed by the Ministries of health to promote rational use of antibiotics.

**Keywords:** Extended Spectrum Beta-lactamases; Prevalence; Gram-negative isolates; Antibiotics susceptibility

## 1. Introduction

Urinary tract infections (UTIs) distress up to 150 million people per annum globally and antibiotics resistance in urinary tract bacteria are growing globally, although it differs according to physical sites and in a straight line to use and misuse of antibiotics [1]. Resilient UTIs if not appropriately treated can lead to impediments such as Pelvic inflammatory Diseases (PID), ectopic pregnancy, abscess formation, Fallopian tube obstruction and associated kidneys triggering nephritis [2]. Impervious organisms have advanced a big task to infectious disease management. It is increasingly being reported in bacteria and is facilitated by genetic movable features like plasmids, transposons, and integrons [3]. Regionally, Africa in contrast with the rest of the world, there is largely a nonexistence of comprehensive data about Extended Spectrum beta-lactamase (ESBLs) manufacturing *Enterobacteriaceae*. Nevertheless, there is enough evidence to highlight the prevalence of ESBLs in Africa [4]. Hospital acquired infections are complex by structural or functional alterations in the urinary tract, weakened renal function or diseases which weaken the immunity, in disparity to community-acquired UTIs [5]. Urinary Tract Infections form the largest single group of hospital acquired infections and account for about 40-50% of the total hospital acquired infections.

Extended Spectrum Beta Lactamases are beta-lactamases capable of hydrolyzing oxyiminocephalosporins and are inhibited by beta-lactamase inhibitors. The occurrence of ESBL manufacturing strains among clinical isolates has been progressively increasing over the past years, causing a constraint of therapeutic choices. These enzymes are plasmid borne and confer multiple drug resistance making UTIs hard to treat. The ESBL manufacturing strains have variable vulnerability rates for aminoglycosides, Fluoroquinolones, and fourth-generation cephalosporins [6-7]. ESBLs were first recognized in 1980s and have progressively blown out throughout the world by hospital acquired routes in hospitals [8-9] and nursing homes as significant reservoirs. Patients admitted to hospitals are more probable reservoirs for resistant bacteria and ultimately, the patients in the community acquire ESBL-manufacturing

[10]. ESBL-producing genes are protected on plasmids 80 kb in magnitude or greater, and most often carry resistance determinants for aminoglycosides, Fluoroquinolones, tetracycline, Cotrimoxazole and even Chloramphenicol, making the bacteria resist a wide diversity of drugs [11]. Mechanisms of drug resistance in gram-negative rods include manufacture of beta-lactamases [12], Amp C lactamases [13], efflux mechanisms and porin deficiency [14]. ESBL manufacturers may show more than one resistance mechanism, further complicating the situation.

The ESBL phenomenon is a new emerging problem that threatens hospitals and other health care centers all over the world. Occurrence and spread of these strains has resulted in treatment failure and disease complications. Recently, new cases have been reported as community-acquired ESBL infections [15]. The existence of an ESBL-manufacturing bacteria in severe infections can end up in treatment disappointment if one of these classes of drugs is used [16]. The cumulative prevalence of infections produced by antimicrobial-resistant bacteria makes empirical therapy of these infections problematic. Furthermore, these infections cause severe complications, particularly in patients with functional or structural abnormalities of the urogenital, patients who have experienced a kidney transplant, patients with polycystic kidneys, and patients with diabetes [17].

Data on epidemiology of UTIs caused by ESBL- manufacturing bacteria are inadequate in published reports in Uganda, yet there is need to monitor and check ESBLs manufacturers among urine isolates especially those associated with hospital acquired infections. Detecting ESBL producer urine isolates helps to improve care of patients mostly the immunocompromised since early detection initiates effective treatment and control measures. Therefore, the aim of the study was to determine prevalence and antibiotics patterns of ESBL producers among Gram-negative urine isolates and their susceptibility at MBN clinical laboratories limited.

## **2. Materials and Methods**

### **2.1 Study design, period and site**

A cross-sectional study was conducted from February to May, 2014, at the department of microbiology-MBN Clinical Laboratories limited, located on plot 28 Nakasero road in Kampala the capital city of Uganda. MBN clinical laboratories limited is a privately owned level three laboratory offering clinical diagnostics and research services to hospitals and research institutions and walk-in patients within and around Kampala city. On a daily basis, a significant number of people from the surrounding zones and nearby districts visits the laboratory for different diagnostic services.

### **2.2 Study population, sample size, sampling technique, and data collection**

All urine samples received for culture at the department of microbiology, MBN clinical laboratories during the study period were included in the study. A total of 200 study samples during the study period were consecutively enrolled by convenient sampling technique.

### 2.3 Sample collection, processing, and culture method

Only mid-stream urine collected by clean catch method in sterile containers were considered. After collection, each sample was transported to the microbiology laboratory at MBN clinical laboratories. The collected samples were inoculated on blood agar and MacConkey agar (Oxoid, Ltd.) and incubated at 35°C. All the plates were incubated aerobically and initially examined for growth after 24 hours, and the ones without growth were further incubated for up to 48 hours.

### 2.4 Isolation, identification, and drug susceptibility testing of bacteria

After obtaining pure colonies, further identifications were done by using the standard microbiological technique, which includes Gram stain, colony morphology, and biochemical tests (Oxoid, Ltd.). Species identification of Bacterial identification was done based on standard bacteriological culture and biochemical tests [18]. The following tests were carried out to confirm the identity of the isolates; Sulphur utilization, Indole production and motility, Urea production, Citrate utilization, Triple sugar iron agar test for sugar fermentation and gas production. Antibiotic susceptibility was tested by the Disc diffusion method according to WHO modified Kirby Bauer disc diffusion method. The inoculum was adjusted to 0.5 Mac Farland standard and then swabbed on Muller Hinton agar plates (MHA). The commercial antibiotics used for gram negative isolates were ; (µg/disc) Ampicillin (10), Gentamycin (10), Ciprofloxacin (5), Co-trimoxazole/Trimethoprim-Sulfamethoxazole, (1.2/23.8), Tetracycline (30), Cefuroxime (30), Cefotaxime (30) or Ceftazidime (30) and Ceftriaxone (30), Imipenem (10), Nitrofurantoin (300), Amoxicillin-Clavulanic acid (20/10).

### 2.5 Extended Spectrum Beta-lactamase detection phenotypic method

Isolates were tested against third generation Cephalosporins (either Cefotaxime or Ceftazidime) using Clinical Laboratory Standard Institute [19] recommended and WHO modified Kirby Bauer disc diffusion method. Zone diameters were interpreted using the revised Clinical Laboratory Standard Institute [19]. Phenotypic confirmatory test for ESBL required use of either Cefotaxime or Ceftazidime alone and in combination with Clavulanic acid. A five millimeter increase in a zone diameter for Ceftazidime tested in combination with Clavulanic acid versus its zone when tested alone was considered an ESBL (e.g. Ceftazidime zone = 16; Ceftazidime/Clavulanic acid zone =21).

### 2.6 Quality control

#### 2.6.1 Quality control the medium:

Sterility of the medium, plates were labeled QC and placed in the incubator for a maximum of three days without inoculating any organism on it and monitored every 24 hours. No growth meant it was sterile and growth implied contamination. For sterile plates, performance testing was done while for those which were not sterile, they were disposed of following the procedures of MBN Clinical laboratory waste management policy. The reliability of the findings was guaranteed by implementing quality control measures throughout the whole processes of the laboratory work. The reference strains used as control were *K. pneumoniae* ATCC 700603, *Escherichia coli* (ATCC 25922)

were inoculated onto the plate and incubated for 24 hours at (35 – 37°C) [18]. Results expected for good quality medium, were considered.

### 2.7 Data analysis

Data were entered and analyzed using SPSS version 18. Simple frequency was applied to determine proportions.

### 2.8 Ethical clearance

Ethical approval was obtained from the Mbarara University of Science and Technology, Faculty of Medicine Ethics and Research Committee. Permission was sought from MBN Clinical Laboratory management to carry out the research.

## 3. Results

A total of 200 urine samples were systematically sampled and received from walk-in patients at MBN clinical laboratory, samples referred from other health facilities in and outside of Kampala and these samples were cultured.

### 3.1 Gram negative urinary pathogens isolated

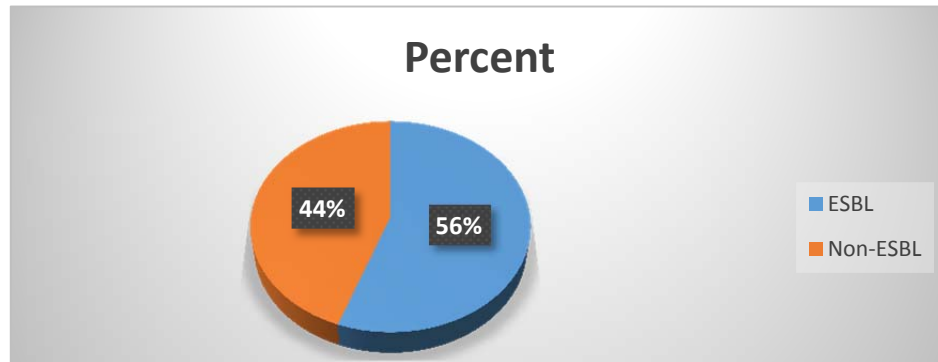
Among the 200 study samples cultured, 45 (22.5%) grew bacteria of clinical significance. Out of the 45 bacterial isolates of clinical relevancy, majority were *Escherichia coli* 28 (62.2%) followed by *Klebsiella pneumoniae* 11 (24.4%) and *C. freundii* 2 (4.4%) while *Enterobacter species*, *Morganella morganii*, *Proteus mirabilis* and *Serratia marcescens* were 1 (2.2%), isolated each (Table 1).

Pathogen	Frequency	Percent	Cumulative Percent
<i>E. coli</i>	28	62.2	62.2
<i>K. Pneumoniae</i>	11	24.4	86.7
<i>C. freundii</i>	2	4.4	91.1
<i>Enterobacter species</i>	1	2.2	93.3
<i>M. Morganii</i>	1	2.2	95.6
<i>Proteus mirabilis</i>	1	2.2	97.8
<i>S. Marcescens</i>	1	2.2	100.0
<b>Total</b>	<b>45</b>	<b>100.0</b>	-

**Table 1:** Gram negative urinary pathogens isolated and identified.

### 3.2. Prevalence of ESBLs in urinary pathogens isolated

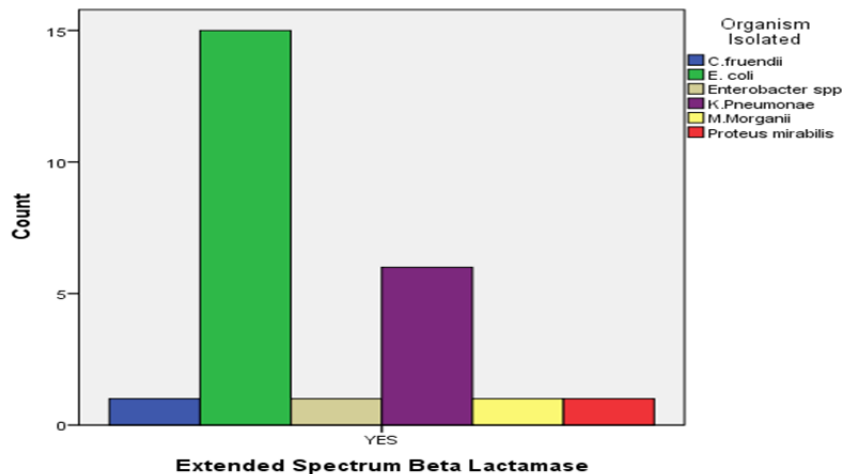
Out of the 45 Gram negative urinary bacterial pathogens 25 (56%) produced ESBLs and 20 (44%) did not produce ESBLs. Therefore, the prevalence of ESBLs among the Gram negative urinary bacterial pathogens was 56%, (Figure 1).



**Figure 1:** Prevalence of ESBL in urinary pathogens isolated.

### 3.3. Distribution of ESBLs among gram negative urinary pathogens isolated

Out of the 25 ESBLs producers, majority of the isolates that produced ESBLs were *Escherichia coli* isolates (15/25; 60%) followed by *Klebsiella pneumoniae* (5/25; 24%) while *Citrobacter freundii*, *Enterobacter species*, *Morganella morganii* and *Proteus mirabilis* each had (1/25; 4%) isolate that produced ESBLs ( Figure 2).



**Figure 2:** Distribution of ESBLs among the Gram negative urinary pathogens isolated.

### 3.4 Antibiotic susceptibility patterns of ESBL producers in urine bacteria isolated

The bacterial isolates showed varied antibiotics susceptibility test results to the selected antibiotics. The isolates were most susceptible to Imipenem 22 (88%), followed by Nitrofurantoin 13 (52%); Cefazidime-Clavulanic acid 10 (40%) and Gentamycin 10 (40%). However some few isolates were less susceptible to Ofloxacin 5 (20%),

Ciprofloxacin 4 (16%), Trimethoprim-Sulfamethoxazole 3 (12%), Ceftriaxone 3 (12%), Ampicillin 1 (4%), Amoxicillin-Clavulanic acid 1 (4%) and Tetracycline 1 (4%). None of the isolates showed susceptibility to Cefuroxime and Ceftazidime (Table 2).

Drug	Susceptibility pattern		
	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Ampicillin	24 (96)	Nil	1 (4)
Amoxicillin-Clavulanic acid	24 (96)	Nil	1 (4)
Tetracycline	24 (96)	Nil	1 (4)
Cefuroxime	23 (93)	2 (8)	Nil
Ceftazidime	23 (92)	2 (8)	Nil
Trimethoprim-Sulfamethoxazole	21 (84)	1 (4)	3 (12)
Ceftriaxone	20 (80)	2 (8)	3 (12)
Ofloxacin	20 (80)	Nil	5 (20)
Ciprofloxacin	20 (80)	1 (4)	4 (16)
Gentamycin	14 (56)	1 (4)	10 (40)
Nitrofurantoin	10 (40)	2 (8)	13 (52)
Ceftazidime-Clavulanic acid	7 (28)	8 (32)	10 (40)
Imipenem	Nil	3 (12)	22 (88)

**Table 2:** Susceptibility pattern of ESBL producing isolates to selected antibiotics n (%).

#### 4. Discussion

The study determined the prevalence and antibiotics susceptibility patterns of ESBL producers among Gram-negative urinary isolates. High prevalence of ESBLs was recorded in this study compared with the prevalence reported by other studies like in India [20], ESBL production was 41% in *Escherichia coli* and 40% in *Klebsiella pneumoniae*. Another study in Nigeria by Olanitola and colleagues in 2007 reported a prevalence of 30%. This study indicated that drug resistant ESBLs producers exist among urine isolated pathogens in our locality. The study showed that ESBL producers were present among urinary gram negative pathogens and they occur at an alarming rate in some species like *Escherichia coli* and *Klebsiella pneumoniae*, prevalence and incidence of ESBL producing strains among urinary isolates have been steadily increasing over the past few years resulting in limitation of therapeutic options; with outbreaks involving ESBL strains which have been reported all over the world, making them emerging pathogens as reported by Ananthkrishnan [21].

Resistant urinary tract infections, if not properly treated, can lead to complications that may result into permanent or temporary infertility, Pelvic Inflammatory Diseases (PID), ectopic pregnancy, abscess formation, Fallopian tube obstruction, epididymitis, orchitis, and the involvement of kidneys causing nephritis [2]. Highest prevalence was

found among the *Escherichia coli* isolates (15/25; 60%) followed by *Klebsiella pneumoniae* (5/25; 24%), while *Citrobacter freundii*, *Enterobacter species*, *Morganella morganii* and *Proteus mirabilis* each had (1/25; 4%). This is due to the fact that ESBL producing isolates are most commonly *Klebsiella species*, and *Escherichia coli* [22]. Other isolates of family *Enterobacteriaceae*, such as *Salmonella species* and *Proteus mirabilis*, and isolates of *Pseudomonas aeruginosa* produce ESBLs but not as common as in *Klebsiella species* and *Escherichia coli* [22].

The urinary Gram negative ESBL producing isolates were most susceptible to Imipenem 22 (88%), followed by Nitrofurantoin 13 (52%); Ceftazidime-Clavulanic acid 10(40%) and Gentamycin 10(40%), due to the fact that *carbapenemases* and Nitrofurantoin exhibit more stability to hydrolysis by ESBLs than the 3rd generation Cephalosporins. A few isolates demonstrated susceptibility to Ofloxacin 5 (20%) and Ciprofloxacin 4 (16%), which is not the case as it was reported by Iroha and others [23-25]. Considering the susceptibility pattern of the Fluoroquinolones (Ciprofloxacin, Ofloxacin) above, newer Fluoroquinolones are unlikely to confer added benefits due to the increased Fluoroquinolones resistance among ESBLs producing isolates in our locality.

The genes encoding the ESBLs production especially CTX-M type *Escherichia coli* are often found to be associated with genetic structures that are genetically linked to class 1 integrons which integrates antibiotic resistance gene cassettes responsible for resistance to  $\beta$ -lactams, aminoglycosides, chloramphenicol, sulphonamides, tetracycline, and ciprofloxacin [26-27]. This could explain the reason of multidrug resistance pattern of the urinary Gram negative ESBL producing isolates investigated in this study.

## 5. Conclusion

The study showed that resistant ESBLs generating bacteria were present among urine isolates. We recommend that ESBLs bacteria isolated in urine be treated grounded on antibiotics susceptibility, continuous surveillance to guide correct treatment for urinary tract infection and to prevent the occurrence of multi drug resistant bacteria. Finally, Ministry of Health develop policies on rational antibiotics use in Uganda.

## 6. Limitation of the Study

We were unable to perform Minimum inhibitory concentration (MIC) and PCR to detect and establish the genes encoding for ESBLs in the isolates.

## Competing Interests

The authors declare that they have no competing interests.

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