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Bacterial Resistance in Urinary Tract Infections: Multidrug Resistant ESBL Producing Gram Negative Uropathogens from Patients

Akosua Bonsu Karikari, Courage Kosi Setsoafia Saba and David Yembilla Yamik

Abstract

Urinary tract infection is one of the most common bacterial infectious diseases encountered in clinical practice. The development and spread of multidrug resistant isolates are of great global health burden; among them, extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae has been a prime concern. This topic describes the resistance patterns of eighty three (83) Gram negative uropathogens to different classes of antibiotics. Bacteria isolates were obtained from patients of all age groups who sought medical attention at a secondary and tertiary hospital in Northern Ghana. Culture and isolation methods employed were the quantitative urine culture on Cysteine Lysine Electrolyte Deficient (CLED) agar and standard biochemical tests. ESBL production was detected using the CLSI recommended phenotypic confirmatory test along with routine antibiotic susceptibility test, adopting the Kirby-Bauer disk diffusion method. Out of 83 isolates, seven (7) Gram negative uropathogens were characterized and ESBLs were detected in 32 of the isolates. *Escherichia coli* was the pathogen with most ESBL positive strains. Generally high and multiple drug resistance were recorded in both ESBL and non-ESBL strains to the empirical drugs, however, ESBL positive strains significantly ($p = 0.000$) showed greater resistance. A notable finding was the appreciable resistance exhibited by ESBL strains to last line treatment drugs that include aminoglycosides and imipenem.

Keywords: Antibiotic resistance, ESBLs, Gram negative uropathogens, UTI, Ghana

1. Introduction

Urinary tract infection (UTI) is a disease of the genitourinary tract that is common in all gender and age groups. Bacteria are the major cause responsible for more than 95% of UTI cases. *Escherichia coli* is the most prevalent causative organism and solely accounts for more than 80% of the infections [1].

In recent times multiple drug resistance among bacteria uropathogens has significantly increased mainly due to the spread of extended spectrum β -lactamases (ESBLs). ESBLs are the enzymes, mostly encoded by plasmids in effect of mutation due to which bacteria show resistance to various β -lactam antibiotics including cephalosporins and monobactams [2]. More than one hundred and fifty (150) ESBL types have been identified and majority of them belong to *class A* enzymes SHV, TEM and CTX-M [3]. These *class A* enzymes hydrolyse penicillin, oxyimino-cephalosporins, and monobactams but not carbapenems or cephamycins and are inhibited *in vitro* by clavulanate [4]. There is a growing apprehension for multidrug-resistant Gram-negative bacteria which produce extended-spectrum β -lactamases [5, 6]. Members of the *Enterobacteriaceae* primarily produce ESBLs particularly *Klebsiella pneumoniae*, *K. oxytoca* and *E. coli*, then again, other Gram-negative organisms including *Salmonella*, *Pseudomonas aeruginosa*, *Proteus spp.* and *Acinetobacter baumannii* have also been named [7].

Immunosuppressed patients with invasive devices, prolonged hospital admissions and long term antibiotic exposure are predisposing factors for colonization or infection with ESBL pathogens [8]. Detecting ESBL producers is a major challenge in clinical settings because of selective pressure caused by heavy use of expanded-spectrum cephalosporins and failures in effective infection control measures in hospitals [9]. Delayed reporting of ESBL producing Gram-negative bacilli is associated with extended clinical admission, increased morbidity and mortality as well as high health care expenditures [4].

Several tests have been recommended for detection of ESBL production *in vitro*. The most frequently used methods include double disc synergy test, combined disc method and E-test. Many automated systems have also been developed for diagnosis while some laboratories use molecular methods for establishing ESBL phenomenon [10].

Still lacking in several healthcare facilities in developing countries including Ghana are laboratories for urine culture and antimicrobial susceptibility testing which obviously lead to unavailable data on ESBLs. Records of prevailing levels of antimicrobial resistance among pathogens are valuable in taking appropriate empirical therapy decisions. Local data of pathogens' susceptibility to antibiotics is virtually absent in most hospitals in Northern Ghana. The purpose of this study was to characterize and screen Gram-negative uropathogens to detect ESBL producers and determine the susceptibility pattern of strains from patients in a secondary and tertiary care hospitals in Northern Ghana. Authors report on the incidence of ESBL-positive Gram negative bacilli in patients presenting with UTI infections in Northern Ghana.

2. Materials and methods

Data was prospectively collected for a period of six (6) months (April 2018 to September 2018) at a tertiary and secondary care hospital in the Northern region of Ghana. A total of 738 non-repetitive mid-stream urine samples were cultured on Cysteine Lysine Electrolyte Deficient Medium (CLED) and isolates were identified by standard laboratory methods [11]. Strains totaling one hundred and ninety (190) were identified and considered clinically relevant which consisted of 107 Gram positives and 83 Gram negative bacilli. In assessing the prevalence of ESBL production among the Gram negative uropathogens, all 83 isolates were further processed for ESBL detection.

Antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines [12].

The drugs used for antibiogram determination were imipenem (10 µg), norfloxacin (10 µg) nitrofurantoin (50 µg), amikacin (30 µg), gentamicin (10 µg), trimethoprim–sulphamethoxazole (co-trimoxazole) (25 µg), ampicillin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), ciprofloxacin (10 µg), augmentin (30 µg), and erythromycin (15 µg). Culture media and all antibiotic discs were sourced from Oxoid. Multiple drug resistance was defined as resistance to three (3) or more classes of antibiotics.

ESBL production was detected by using the CLSI recommended phenotypic confirmatory test along with routine antibiotic susceptibility testing. This was performed with ceftadizime (CAZ 30 µg) and cefotaxime (CTX 30 µg) discs alone and in combination with clavulanic acid (CAZ/CLA 30/10 µg). A ≥ 5 mm increase in zone size of the combined ceftazidime and clavulanic acid was considered as confirmation of ESBL production [12]. All the recovered Gram negative bacteria were subjected to ESBL screening although CLSI phenotypic confirmatory test endorses *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. *Escherichia coli* ATCC 25922 was used as negative control for ESBL production due to unavailability of a positive control strain. All susceptibility testing was performed on Mueller-Hinton agar using 0.5 McFarland suspension from overnight cultures, followed by incubation at 35°C for 16 to 18 hrs.

SPSS version 20 was used to analyze the data. Descriptive statistics including frequencies and percentages were used. Pearson chi-square test at 95% significant level was conducted to determine associations between categorical outcome variables. A two tailed p-value of <0.05 was considered statistically significant. Approval for the study was obtained from the Ethical Review Committee of the Tamale Teaching Hospital.

3. Results

Out of the 738 urine screened, 190 were considered significant bacteraemia. The 190 uropathogens comprised 107 Gram positives and 83 Gram negatives. Of the 83 Gram negative isolates screened, 32 (38.6%) were positive for ESBL production and *E. coli* was the predominant (37.5%) ESBL producing pathogen. About 81% (26) of the ESBL strains came from in-patients and approximately 38% were recovered from patients who were 60 years and above. ESBL-positive strains were significantly ($P = 0.002$) greater in female isolates (**Table 1**).

	No. ESBL positive patients	Percentage (%)	P-value
Age			
< 20	2	6.3	0.000
20–39	11	34.4	
40–59	7	21.4	
60 and Above	12	37.5	
Sex			
Male	15	46.9	0.002
Female	17	53.1	
Total	32	100.0	

Table 1.
Gender and age distribution of patients with ESBL positive strains.

Comparing ESBL and non-ESBL strains, the difference in resistance pattern was significant, $p = 0.000$. ESBL producing strains showed up to 50% resistance to aminoglycosides (gentamicin and amikacin) and about 22% to imipenem while resistance of non-ESBLs were below 10% to aminoglycosides (gentamicin and amikacin) and approximately 10% to imipenem. The non-ESBL strains were highly resistant (70–90%) to only ampicillin, erythromycin, nitrofurantoin and tetracycline but ESBL strains generally showed high resistance (50–100%) to almost all the drugs, with exception to amikacin and imipenem where resistance was below 30%. Resistance to cefepime was about 84% among ESBL strains and 19.6% in the non-ESBL strains (**Table 2**).

Multidrug resistance was a common occurrence in the ESBL strains with approximately 68% of them being resistant to six (6) or more antibiotics but only 15.7% of the non-ESBL strains showed this particular phenomenon, **Table 3**. All ESBL positive and negative strains of *E. coli*, *Klebsiella* and *Salmonella* showed 100% multiple drug resistance (**Table 4**).

Resistance of β -lactamase producing *E. coli* strains to the quinolones (ciprofloxacin, norfloxacin) were up to 83%, however the non- β -lactamase producing strains showed resistance of about 26–30% to the same class of drugs. Also, against the 3rd generation cephalosporins (ceftriaxone, ceftazidime and cefotaxime) the β -lactamase producers showed resistance of 83–100%, but resistance was between 21 and 26% among the non- β -lactamase *E. coli* strains (**Table 5**).

Klebsiella strains that were β -lactamase positive showed resistance of up to 44% to the quinolones while strains that did not produce β -lactamase had resistance of about 33%. Resistance to the aminoglycosides were 22–66.7% in the ESBL strains and 0% in the non-ESBL strains. Against the 3rd generation cephalosporins, the ESBL strains showed resistance of 88–100% as the non-ESBL strains had resistance of 16–33% (**Table 5**).

Antibiotic	% ESBL Strains (n = 32)	% Non-ESBL Strains (n = 51)	P-Value
Cefoxitin	53.1	39.2	0.000
cefepime	84.4	19.6	
Ciprofloxacin	59.4	21.6	
Norfloxacin	62.5	23.5	
Amikacin	21.9	7.8	
Gentamicin	50.0	7.8	
Ampicillin	96.9	90.2	
Augmentin	62.5	31.4	
Tetracycline	78.1	70.6	
Ceftriaxone	84.4	23.5	
Ceftazidime	93.8	27.5	
Nitrofurantoin	75.0	72.5	
Chloramphenicol	59.4	54.9	
Erythromycin	75.0	86.3	
Cotrimoxazole	78.1	64.7	
Imipenem	21.9	9.8	
Cefotaxime	100.0	31.4	

Table 2.
Resistance pattern of ESBL and non ESBL strains of UTI patients.

Multidrug Resistance	ESBL Producers	Non ESBL Producers	P-Value
	No. of Resistant Strains (%)	No. of Resistant Strains (%)	
3 Classes of antibiotics	3(9.7)	27(52.9)	0.000
4 Classes of antibiotics	2(6.5)	12(23.5)	
5 Classes of antibiotics	5(16.1)	4(7.8)	
6 Classes of antibiotics	7(22.6)	2(3.9)	
≥7 Classes of antibiotics	14(45.2)	6(11.8)	
Total	31(100.0)	51(100.0)	

Table 3.
Multidrug resistance of ESBL and non ESBL strains.

Organism	ESBL Producers		Non- ESBL Producers		P-Value
	Isolates (n)	MDR (%)	Isolates	MDR (%)	
<i>E.coli</i>	12	12(100.0)	23	23(100.0)	0.204
<i>Salmonella</i>	2	2(100.0)	2	2(100.0)	
<i>Klebsiella</i>	9	9(100.0)	6	6(100.0)	
<i>Enterobacter</i>	4	4(100.0)	12	12(100.0)	
<i>Serratia</i>	4	3(75.0)	7	7(100.0)	
<i>Pseudomonas</i>	0	0(0.0)	1	1(100.0)	
<i>Proteus</i>	1	1(100.0)	0	0(0.0)	

Table 4.
Multidrug resistance rate of uropathogens.

Enterobacter producing ESBL showed 75% resistance to the quinolones while 0% resistance was observed in the non-ESBL strains and to the aminoglycosides resistance were 25–50% in the ESBL strains and 0% among the non-ESBL strains (Table 5).

ESBL-positive *E. coli*, *Klebsiella* and *Enterobacter*, showed respective resistivity of 25%. 33.3% and 25% to Imipenem while in the non-ESBL strains, resistance were 8.7% in *E. coli* and 0% each in *Klebsiella* and *Enterobacter* sp. (Table 5).

4. Discussion

In recent times antimicrobial resistance has been acknowledged as a major public health problem worldwide with developing countries reporting more worrying trends. The emergence and rapid dissemination of multiple drug resistant pathogens including ESBL producing Enterobacteriaceae have made management of hospital and community acquired infections caused by these strains difficult. The prevalence of ESBL producing pathogens greatly differs from country to country and also within country. Prevalence ranging from below 1% to more than 70% have been documented globally [13].

We identified seven (7) species of Gram negative uropathogens and detected ESBLs in 32 (38.6%) out of 83 uropathogens recovered from patients reporting to hospitals in Northern Ghana. Among the Seven (7) Gram negative species screened for ESBL production, *E. coli* was the pathogen with the most (37.5%) ESBL positive strains which is comparable to reported rates from India [14], Turkey [15]

Organism	Antibiotics																
	FOX	FEP	CIP	NOR	AK	GEN	AMP	ACA	TE	CRO	CAZ	NIT	CHL	ERY	COT	IPM	CTX
ESBL strains																	
<i>E. coli</i> (12)	33.3	91.7	83.3	83.3	25.0	41.7	100.0	58.3	100.0	83.3	100.0	66.7	50.0	75.0	91.7	25.0	100.0
<i>Salmonella</i> (2)	100.0	50.0	0.0	0.0	0.0	100.0	100.0	50.0	50.0	100.0	100.0	100.0	100.0	100.0	50.0	0.0	100.0
<i>Klebsiella</i> (9)	77.8	77.8	44.4	33.3	22.2	66.7	100.0	66.7	55.6	88.9	88.9	88.9	77.8	77.8	66.7	33.3	100.0
<i>Enterobacter</i> (4)	75.0	100.0	75.0	75.0	25.0	50.0	100.0	75.0	100.0	100.0	100.0	50.0	25.0	100.0	100.0	25.0	100.0
<i>Serratia</i> (4)	25.0	100.0	50.0	75.0	25.0	25.0	75.0	50.0	50.0	50.0	100.0	75.0	50.0	50.0	50.0	0.0	100.0
<i>Proteus</i> (1)	0.0	0.0	0.0	100	0.0	0.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	0.0	100.0	0.0	100.0
Non-ESBL strains																	
<i>E.coli</i> (23)	21.7	21.7	26.1	30.4	17.4	4.4	87.0	17.4	87.0	26.1	21.7	56.5	56.5	91.3	78.3	8.7	26.1
<i>Salmonella</i> (2)	100.0	0.0	100.0	100	0.0	100.0	100.0	100.0	100.0	100	100.0	100	100.0	100	100.0	50.0	100.0
<i>Klebsiella</i> (6)	50.0	16.7	33.3	33.3	0.0	0.0	100.0	50.0	66.7	33.3	33.3	66.7	50.0	83.3	83.3	0.0	16.7
<i>Enterobacter</i> (12)	41.7	8.3	0.0	0.0	0.0	0.0	83.3	16.7	58.3	0.0	16.7	83.3	33.3	91.7	33.3	0.0	16.7
<i>Serratia</i> (7)	57.1	42.9	14.3	14.3	0.0	14.3	100.0	57.1	28.6	14.3	42.9	100.0	71.4	71.4	42.9	14.3	57.1
<i>Pseudomonas</i> (1)	100.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100	0.0	100.0	100.0	0.0	100.0	100.0	100.0

FOX, Cefoxitin; FEP, Cefepime; CIP, Ciprofloxacin; NOR, Norfloxacin; AK, Amikacin; GN, Gentamycin; AMP, Ampicillin; ACA, Augmentin; TE, Tetracycline; CRO, Ceftriaxone; CAZ, Ceftazidime; NIT, Nitrofurantoin; CHL, Chloramphenicol; ERY, Erythromycin; COT, Co-trimoxazole; IPM, Imipenem; CTX, Cefotaxime.

Table 5.
Resistance pattern of non ESBL and ESBL producing uropathogens.

and Saudi Arabia [16], but lower than a report from Egypt [17]. Moreover, in Africa, the prevalence of ESBL-producing *E. coli* has been found to range from 35 to 65% [18]. From literature, ESBLs are produced primarily by members of the Enterobacteriaceae and other pathogens including *Acinetobacter baumannii*, *Proteus* spp., *Pseudomonas aeruginosa* and *Salmonella* spp. [19] but detection is more frequent in *E. coli* and *Klebsiella* species [20].

ESBL positivity was significant in females (0.002) compared to the male gender. In contrast, authors from India [14] and Israel [21] have reported male prevalence citing male gender as a risk factor for community-acquired ESBL-positive UTI [21]. Our study however involved in-patient and OPD cases of UTI with 81% of strains coming from in-patients, which possibly could account for the difference in results. Besides, females are often beset with UTI due to settlement of colonic Gram negative bacteria on the urethra as a consequence of a short urethra and its closeness to the anus. The gender of a patient, according to Magliano and colleagues is one of the risk factors of UTI [22].

Age group 60 and beyond (37.5%) were mostly found with ESBL positive strains. Several studies have indicated age over 60 years to be an associated risk factor for community-acquired infections with ESBL-producing microorganisms in adults [21, 23, 24]. This age bracket is putatively prone to infections, which is expected to make them consume antibiotics in greater quantities that could ultimately contribute to drug resistance.

Therapeutic challenge is allied with ESBL-producing strains due to low susceptibility to variety of β -lactams, including third generation cephalosporins as well as the possibility for plasmid mediated quinolone and carbapenem resistance. The ESBL isolates showed high rates of resistance to all studied antibiotics with exception to amikacin and imipenem, where resistance to these drugs were 21.9% each. The 21.9% may be considered high when compared to documented rates in different geographical regions where susceptibility to imipenem was 100% [13, 25, 26]. The resistant pattern of our study isolates reaffirms accounts of low susceptibility of ESBL strains to third generation cephalosporins and other β -lactam drugs. Respective resistances of ESBL strains to the third generation cephalosporins; cefotaxime, ceftazidime and ceftriaxone were 100%, 93.8% and 84.4% and 31.4%, 27.5% and 23.5% were for non-ESBL strains and the difference was statistically significant (0.000). Similarly, susceptibility of ESBL strains to ampicillin and augmentin were low (62.5–96.9% resistance). The non-ESBL strains however showed equally high resistance to ampicillin (90.2%) and rather lower resistance (31.4%) to augmentin.

Antibiotics including quinolones (ciprofloxacin, norfloxacin), cefepime, tetracycline, cotrimoxazole had ESBL positive strains exhibiting greater resistance to them as opposed to non-ESBL strains with a significant difference. Quite the reverse occurred with erythromycin where non-ESBL isolates resistance (86.3%) was significantly more than the ESBLs (75.0%); but almost equal resistance was observed in ESBLs (75.0%) and non-ESBL strains (72.5%) to nitrofurantoin.

A notable finding of this research was the high resistance of both ESBL and non-ESBL strains to first line and empirical drugs of UTI. Multiple drug resistance of 100% was observed in six (6) of the seven (7) uropathogens identified and close to 66% of the ESBL strains were resistant to six (6) or more antibiotics. Additionally, resistance to aminoglycosides which have reportedly been low [13, 27–29] and considered a treatment option for complicated UTI was not effective against our ESBL strains, with up to 50% of strains showing resistance. Limiting the use of a group of antibiotics could lead to over prescription of other classes resulting in a surge of resistance in the oversubscribed drugs. The rampant pathogen resistant reports to frequently used and affordable drugs are gradually putting pressure on the last

line class of drugs including the carbapenems. A review of antimicrobial resistance studies in Ghana have shown a steady rise in resistance to classes of antibiotics such as aminoglycosides and carbapenems (personal review, unpublished) which previously were very effective and rarely suffered pathogen resistance. This research documented 21.9% resistance from ESBL strains and almost 10% from Non-ESBL isolates to imipenem. This clinical warning of increased resistance to last line antibiotics and high MDR records prompt an immediate need to formulate strategic policy initiatives to reduce their emergence and spread. Regulating the emergence and spread of ESBL organisms in hospitals require a blend of antimicrobial stewardship and effective infection control compliance in hospitals. Consistent monitoring of regional and national surveillance data of the common infectious pathogens besides screening of ESBL producers is of prime importance in controlling the rise in multi-drug resistant pathogens.

5. Conclusion

The study found *E. coli* with most ESBL producing strains. Multiple drug resistance was a common occurrence; in almost all the Gram negative uropathogens characterized, 100% resistance was recorded. ESBL strains generally showed greater resistance than the non-ESBL strains particularly to the cephalosporins and β -lactam antibiotics. However resistance to UTI empirical drugs and other commonly used antibiotics were alike in both ESBL and non-ESBL strains as low susceptibility was observed. An important finding was the considerable resistance of the ESBL strains to the aminoglycosides and imipenem which are last line treatment drugs. The results of the study clearly indicate the need for antimicrobial stewardship and enhanced infection control measures in our hospitals. Routine screening of ESBLs in our hospitals is highly recommended since appropriate antimicrobial therapy can only commence with early detection of these strains.

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