ORIGINAL RESEARCH ARTICLE

Prevalence of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella* species from Urinary Specimens of Children attending Friendship International Children's Hospital

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Abstract

Extended-spectrum β-lactamase producing *E. coli* and *K. pneumoniae* is a serious threat to the patients. These organisms are major extended spectrum beta lactamase (ESBL) producers. The objective of this study was to determine the prevalence of Extended spectrum β- lactamase producing strains of *Escherichia coli* and *Klebsiella* spp isolates from the urine sample of children visiting International Friendship Children Hospital. During the seven months, between June 2016 to December 2016, 1018 mid-stream urine samples(MSU) were collected from patients suspected of having UTI. The samples were investigated by conventional semi-quantitative culture technique and identification of *E. coli* and *Klebsiella spp.* was done by microscopy and biochemical test. Antibiotic susceptibility test of isolates was performed by modified Kirby Bauer Disc diffusion test. ESBL screening test was done by using 3rd generation Cephalosporin and confirmation done by combination disc diffusion method. Out of total 1018 MSU samples investigated, 200(19.64%) isolates of *E. coli* and 28(2.7%) isolates of *Klebsiella spp.* making a total of 228(22.39%) were found to cause significant bacteriuria. 76(33.33%) isolates, from those causing significant bacteriuria, were Multi-drug resistant organisms. Out of 228 isolates, 54(23.68%) were ESBL producers, that includes 51(25.5%) *Escherichia coli* and 3(12.5%) *Klebsiella pneumoniae.* ESBL producers were more common in in-patient (36.17%) than out-patient (20.44%). Most of the ESBL producers were resistance to amoxicillin, followed by Cotrimoxazole and Ciprofloxacin respectively. They were highly sensitive to Imipenem, Tigecycline, Amikacin, Piperacillin-Tazobactam, and Nitrofurantoin. High prevalence of ESBL producing *E. coli* and *Klebsiella pneumoniae* was found among children. Regular and routine monitoring of ESBL producing isolates is essential.

Key words: Urinary tract infection(UTI), Extended spectrum beta lactamase(ESBL), *E. coli*, *Klebsiella*
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Introduction

Urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging in severity from asymptomatic which is carrier status in the urine to symptomatic acute infection of the kidney with resultant sepsis [1]. The clinical symptoms of UTI usually include frequency, dysuria, pyuria, suprapubic tenderness, back pain, fever and urgency [2]. The most common uropathogenic Gram negative bacteria are *Escherichia* coli and *Klebsiella pneumoniae* [3].

Extended-spectrum β lactamases (ESBLs) are a group of enzymes with the ability to hydrolyze and cause resistance to the oxyimino-cephalosporins (i.e.cefotaxime, ceftazidime, ceftriaxone, cefuroxime and cefepime) monobactams (i.e. aztreonam) [4]. Beta-lactamases are among the most heterogeneous group of resistance enzymes. Despite a significant amount of amino acid sequence variability, beta-lactamases share a common overall topology [5]. There are several ESBLs genotypes. These are SHV, TEM, and CTX-M [6]. Other clinically important genotypes include OXA, VEB, PER, BES-1, BEL-1, SFO-1, TLA, and IBC [7]. "Classical" ESBLs are derived from TEM and SHV enzymes whereas "Non-Classical" ESBLs are derived from enzymes other than TEM or SHV. 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1 [8]. Based on different combinations of changes, currently, 195 TEM-type enzymes have been described. TEM-2, the first variant described, differed from TEM-1 through the substitution of a lysine for a glutamine at position 39 [6]. A shift in the distribution of different ESBLs has recently occurred in Europe, with a dramatic increase of CTX-M enzymes over

TEM and SHV variants [9]. Since 2000, *E. coli* producing CTX-M β-lactamases have emerged worldwide as an important cause of community-onset urinary tract infections (UTIs) and this has been called the CTX-M pandemic [10]. These enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates (e.g. ceftazidime, ceftriaxone, or cefepime) [7]. Rather than arising from mutation, they represent examples of plasmid acquisition of beta-lactamase genes normally found on the chromosome of *Kluyvera* species, a group of rarely pathogenic commensal organisms [9]. In France, a novel derivative of OXA-10 (numbered OXA-28) was found in a *P. aeruginosa* isolate [11]. The rapid increasing rate of ESBL production among *Enterobacteriaceae*, mainly *E. coli* and *Klebsiella pneumonia*, have created a serious global public health problem [12]. Availability of limited treatment options for the infections caused ESBL producing bacteria makes the treatment very difficult and often results into treatment failure [13]. Scant number of studies had been reported from Nepal assessing the ESBL producers among *E. coli* and *Klebsiella spp* in children. Hence the study was carried out to determine the prevalence of ESBL producing *Escherichia coli* and *Klebsiella spp* isolates from the urine samples of children.

Materials and Methodology

Sample collection and identification of bacteria

The cross-sectional study was carried out in Pathology Department of International Friendship Children Hospital, Maharajgunj, Kathmandu from June 2016 to December 2016. Patients under the age of 13 years or their guardians visiting the Pathology Department were directly interviewed for his/her clinical history during the sample collection. During the study period, 1018 midstream urine samples were collected and processed according to the standard laboratory methods by Forbes et al [14]. Semi-quantitative culture technique was used to detect the presence of significant bacteriuria. The bacterial culture was done on MacConkey Agar (MA) and Blood Agar (BA) and incubated overnight at 37°C for isolation of pure culture. Diagnosis of UTI was made when there were colony count exceeding 10^5 cfu/ml of urine specimen. The isolates were identified based on morphology, gram's staining, motility, and standard biochemical tests as described in Forbes et al [14].

Modified Kirby-Bauer's disc diffusion method was employed for the antibiotic susceptibility test of potential pathogenic isolates as per standard technique on Muller Hilton Agar [15]. Nitrofurantoin(300mcg), Amoxicillin (30mcg), Cotrimoxazole (25 mcg), Ciprofloxacin (5mcg), Ceftriaxone (30 mcg), Ceftazidime (30 mcg), Gentamicin (10 mcg), Imipenem (10 mcg) and Meropenem (10 mcg) were used for antimicrobial susceptibility testing.

Screening of ESBL producing strains of *E. coli* and *K.pneumoniae*

The screening test for the production of ESBL was performed using both Ceftazidime (CAZ) (30µg) and Ceftriaxone (CTR) (30µg) disks. If the zone of inhibition was less than or equal to 22mm for CAZ and/or less than or equal to 25mm for CTR, the isolate was considered a potential ESBL-producer as recommended by CLSI [16].

Phenotypic confirmation of ESBL production

Susceptible screened ESBL producers were subjected to combined disk test as recommended by the CLSI [16]. Combination disk method used for the confirmation of ESBL-producing strains in which CAZ (30µg), alone and in combination with Clavulanic acid (CA) (10µg) were used. After incubating overnight at 37°C, ≥ 5 mm increase in the zone diameter for either antimicrobial agent which was tested in combination with Clavulanic acid (CAC) versus its zone when tested alone, was interpreted as positive for ESBL production.

Results

Table 1: Microbiological profile of bacterial isolates

S.N	Bacterial isolates	Number	Percent
1	*Escherichia coli*	200	82.30%
2	*Klebsiella pneumoniae*	24	10%
3	*Klebsiella oxytoca*	4	1.64%
4	*Citrobacter* spp	3	1.23%
5	*Pseudomonas aeruginosa*	2	0.82%
6	*Acinetobacter*	2	0.82%
7	*Staphylococcus aureus*	8	3.29%
	Total	**243**	**100%**

Out of the total 1018 mid-stream urine samples, 243(23.87%) samples were found to have a significant growth. Out of 243 culture positive cases, *Escherichia coli* (200) (82%) was found to be

the most common isolates followed by *Klebsiella pneumoniae* (24) (10%), *Klebsiella oxytoca* (4) (1.65%), *Citrobacter* species (3) (1.15%), *Pseudomonas aeruginosa* (2) (0.85%), *Acinetobacter* species (2) (0.85%) and the gram positive isolates, *Staphylococcus aureus* (8) constitutes (3.30%) (**Table 1**). Most of the patient were of age group 1-5 (60.96%) followed by age <1 (18.42%) (**Table 2**).

Table 2: Demographic distribution of *E. coli* and *Klebsiella* spp

Age group	Sex of patient		Total
	Male (%)	Female (%)	
<1 year	12 (28%)	30 (72%)	42(18.42%)
1-5	49 (35%)	90 (65%)	139(60.96%)
6-10	11 (34.3%)	21 (65.6%)	32(14.03%)
11-13	3 (20%)	12 (80%)	15(6.57%)
Total	75	153	228

Out of 200 *E. coli* isolates, 169(84.5%) isolates were sensitive to Nitrofurantoin followed by gentamycin 146(73%) and cotrimoxazole 110(55%). More than 30% isolates were resistant to third generation cephalosporins i.e Ceftriaxone and Ceftazidime (**Table 3**).

Table 3: Antibiotic susceptibility pattern of *Escherichia coli*

Antibiotics	Susceptibility pattern (n=200)		
	Sensitive (%)	Intermediate (%)	Resistance (%)
Amoxicillin	14 (7)	23 (11.5)	163 (81.5)
Ciprofloxacin	91 (45.5)	31 (15.5)	78 (39)
Cotri-moxazole	110 (55)	22 (11)	68 (34)
Nitrofurantoin	169 (84.5)	20 (10)	11 (5.5)
Gentamycin	146 (73)	21 (10.5)	33 (16.5)
Ceftriaxone	108 (54)	30 (15)	62 (31)
Ceftazidime	98 (49)	34 (17)	68 (34)

Out of total 24*K. pneumoniae* isolates, 19(79.2%) were sensitive towards Gentamycin, followed by Nitrofurantoin 17 (71%), Ciprofloxacin 16 (67%) and Ceftriaxone 16 (67%) (**Table 4**).

Table 4: Antibiotic susceptibility pattern of *K. pneumoniae* isolates.

Antibiotics	Susceptibility pattern(n=24)		
	Sensitive (%)	Intermediate (%)	Resistance (%)
Amoxicillin	0 (0)	0 (0)	24 (100)
Ciprofloxacin	16 (67)	4 (17)	4 (17)
Cotrimoxazole	14 (58)	3 (13)	7 (29)
Nitrofurantoin	17 (71)	1 (4)	6 (25)
Gentamycin	19 (79.2)	3 (12.5)	2 (8.3)
Ceftriaxone	16 (67)	2 (8)	6 (25)
Ceftazidime	14 (58)	4 (17)	6 (25)

Among total 4 *K. oxytoca* isolates, all 4 were resistant to Amoxicillin, howeversensitive to Nitrofurantoin., 3 (75%) of them were sensitive to to Cotrimoxazole, followed by ciprofloxacin. 68 (34%) of *E. coli* isolates, 6(25%) of *Klebsiella pneumoniae* isolates and 2 (50%) of *Klebsiella oxytoca* isolates, making a total of 76(33.33%) isolates were Multi-drug resistant (**Table 5**).

Table 5: Distribution of MDR isolates

Bacterial isolates	Total	MDR (%)
Escherichia coli	200	68 (34%)
Klebsiella pneumoniae	24	6 (25%)
Klebsiella oxytoca	4	2 (50%)
Total	**228**	**76(33.33%)**

Out of 228 isolates, 61 isolates gave ESBL screening test positive. 51 isolates of *E. coli* and 3 isolates of *Klebsiella pneumoniae* making a total of 54(23.68%) were confirmed as ESBL producers. None of *K. oxytoca* was ESBL producer (**Table 6**).

Table 6: Detection of ESBL by combination disk method.

Organism	Significant growth	Screening test positive	Confirmatory test(combination disk method)
			Increase in diameter ≥5mm
Escherichia coli	200	57	51(25.5%)
Klebsiella pneumonia	24	4	3(12.5%)
Klebsiella oxytoca	4	-	-
Total	228	61	54(23.68%)

Out of 75 isolates from male, 12(16%) were ESBL producer and out of 153 isolates from female 42(27.54%) were ESBL producer. More female was infected as compared to male, also chi-square test suggests a significant association in between sex of a patient and ESBL producers(p-value<0.05) (**Table 7**).

Table 7: Gender wise distribution of ESBL producing isolates.

Sex	ESBL producer	ESBL non-producer	Total	p-value
Male	12(16%)	63(84%)	75	<0.05
Female	42(27.45%)	111(72.54%)	153	
Total	54(23.68%)	174(76.31%)	228	

Among culture positive cases, 47 were from In-patient and remaining 181 were from out-patient. Among 47 In-patient, 17(36.17%) was found to be ESBL positives. Similarly, among 181 Out-patient, 37(20.44%) was found to be ESBL positives (Table 8). All 51 ESBL producing isolates of *E. coli* were sensitive to Imipenem, Piperacillin-tazobactam, Tigecycline and Amikacin. 94.2%and 92.2% of *E. coli* were resistant to Ceftriaxone and Ceftazidime, respectively.

Table 8: Department wise distribution of ESBL producers.

	In-patients	Out-patients	Total	p-value
ESBL producer	17(36.17%)	37(20.44%)	54	<0.05
ESBL non-producer	30(63.82%)	144(79.55%)	174	
Total	47	181	228	

Furthermore, 88.2% and 31.4% of *E. coli* were resistant to Cefepime and Meropenem, respectively (**Table 9**). All of the 3 ESBL producing *K. pneumoniae* isolates were sensitive to Imipenem, Meropenem, Piperacillin-tazobactam, tigecycline, and Amikacin. All of the isolates were resistant to Cefepime and Amoxicillin.

Table 9: Antibioitic susceptibility pattern of ESBL producing strain of *E. coli*

Antibiotics	*E. coli* (n=51)			
	Sensitive	Sensitive %	Resistance	Resistance %
Amoxicillin	-	-	51	100
Ciprofloxacin	13	25.5	38	74.5
Cotrimoxazole	13	25.5	38	74.5
Nitrofurantoin	48	94.1	3	5.9
Gentamicin	32	66.7	19	37.3
Ceftriaxone	3	5.9	48	94.1
Ceftazidime	4	7.8	47	92.2
Imipenem	51	100	0	0
Meropenem	35	68.6	16	31.4
Cefepime	6	11.8	45	88.2
Piperacillin	51	100	-	-
Tigecycline	51	100	-	-
Amikacin	51	100	-	-

Discussion

Out of 1018 mid-stream urine sample, 243 (23.87%) showed significant growth. Similar result was obtained by Bhandari **(**2013) where growth positivity was found to be 23.36% [17]. Similarly, the study carried out in India by Niranjan *et al* (2014) yielded 18.5% significant growth [18]. However, our result is low as compared to that reported from South Africa (51%) by Habte *et al* (2009) [19]. A study conducted in Nepal by Bhatta *et al* (2013), demonstrated similar result with 27.3% significant growth [20]. The rate of infection found in female patients was 163/684 (23.83%) and in male, the rate of infections was found to be 80/354 (22.59%). There wasn't any huge difference between rate of infection in male and female children patient. This result is in contrast to the earlier studies by Thakur *et al* (2013) where 56.64% female and 43.36% male patient were infected [21]. The growth positivity with 33.5% among female patients and 23.7% in male patients was observed in a similar study by Baral *et al* (2012)[22].

The predominant isolate was *E. coli* (82.30%) followed by *Klebsiella* species (11.53%) and *Staphylococcus aureus* (3.29%). *E. coli* is a predominant isolate, because *E. coli* can bind to the glycol-conjugate receptor of the uroepithelial cells of human urinary tract so it can initiate infection itself. *E. coli* is isolated in 90% of infection and strains are characterized by presence of unique virulence determinant the pilus (Gal-Gal) receptor [23]. Similar result was obtained by **other studies** [24, 25].

E. coli was found to be resistant towards amoxicillin (81.5%), Co-trimoxazole (35%), and Ciprofloxacin (38%). In our study, 25% of *K. pneumoniae* were resistant to both Ceftriaxone and Ceftazidime. Other studies from Nepal reported that the resistance rates of *K. pneumoniae* to third-generation Cephalosporin were between 20% to 75% [26,27].

Likewise, 25% and 8.3% of *K. pneumoniae* were found resistant to Nitrofurantoin and Gentamicin, respectively. All *K. pneumoniae* strains were resistant (100%) to Amoxicillin, while resistance rate for *E. coli* was 81.5%. Similar result was observed in a study carried out at Madagascar, where 80% of the *E. coli* isolates were resistant to amoxicillin [28]. In our study, *E. coli* showed a high resistance rate 68% to Co-trimoxazole whereas the *K. pneumoniae* showed resistance rate 30% to Co-trimoxazole. A comparable resistance rate of 80% and 45% to Cotrimoxazole was shown among ESBL producing *E. coli* and *K. pneumoniae* isolates in a study conducted in Iran [29]. Bazzaz *et al* (2009) reported ESBL-producing isolates of *K. pneumoniae* and *E. coli* as 59.2% [30].

In this study, 17% isolates of *K. pneumoniae* were resistant to Ciprofloxacin. Resistant to fluoroquinolones is due to the result of alterations in target enzyme (DNA gyrase and topoisomerase IV) and because of change in drug entry and efflux [31]. *Klebsiella pneumoniae* sensitivity towards Gentamicin was found in 79.2% which was significantly higher than that was reported in Karanchi, Pakistan where a resistance rate for gentamicin was 46.7% [32]. However, the rate was only slightly higher than to the findings from previous study done in Nepal where the sensitivity was 72.9% [22]. These significant variations may be attributed to selective pressures

by drugs in different regions. Resistance of aminoglycosides is done by the enzymes that cause modification of drug by phosphorylation, acetylation or adenylation and less or more by other methods [33].

In our study, 31.4% of ESBL positive *E. coli* was resistant to Meropenem, whereas all isolates were sensitive to Imipenem which is similar to the study done in Peshawar, Pakistan [34]. The emergence of carbapenem resistance in *K. pneumoniae* is typically attributed to the production of *Klebsiella pneumoniae* carbapenemase (KPC) [35].

We found the 76 (33.33%) isolates of *E. coli* and *Klebsiella spp* were multidrug resistant. Similar findings were observed in the study done by Tuladhar *et al* (2003), in a hospital in Kathmandu, where 35.21% of bacterial strain were MDR [36]. But the result was in contrast to the study by Upadhaya *et al* (2013) where 48% were MDR [37]. The prevalence of MDR varied among different studies and outcome of the prevalence may depend on various factors such as MDR criterion, how the antibiotics are used, and organism encoding multiple resistance gene which is becoming more prevalent.

Out of 228 isolates, 54 (23.68%) were ESBL producer. Our finding was lower in comparison to the study conducted by Dahal *et al* (2016), in a community hospital of Kathmandu that reported 47.75% as ESBL producer [25]. A study done in India reported nearly 40% of urinary isolates of *E. coli* and *K. pneumoniae* were ESBL positive [38]. Our result was similar to the study done by Poudyal (2010) where 25.7% ESBL producer were isolated from urine sample [39]. However, our result showed higher prevalence as compared to the study by Logan *et al* (2014) in the United States with ESBLs representing only 0.28% of all *E. coli*, *K. pneumoniae*, and *P. mirabilis* in children from 1999 to 2001 and later increasing to 0.92% of all isolates in 2010–2011 [40]. The occurrence of ESBLs among clinical isolates varies greatly from country to country, among the hospitals, within the country.

In our study, 25.5% of *E. coli* isolates and 12.5% of *Klebsiella pneumoniae* isolates were ESBL producer. These findings were comparable to the study done by Chander and Shrestha (2013) in Nepal tertiary hospital in which 13.51% of *E. coli* and 16.55% of *Klebsiella pneumoniae* were ESBL producers [41]. ESBL occurrence among *E. coli* and *Klebsiella* is of great concern since infections caused by these bacteria are very common and resistance of the organism may be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, they also spread easily, are pathogenic and efficient at acquiring and disseminating resistance plasmid [42]. More female (27.45%) were infected as compared to male (16%). Similar result was obtained by Bhandari (2013) [17].

Though some of our results were very much contrast with other studies, the prevalence rate of ESBL producing isolates in our study was considered significant and high. The higher number of ESBL producers might be due to more reliance on third generation Cephalosporins to treat Gram negative infections and unscrupulous hospital antibiotic policy. Moreover, high prevalence of ESBL isolates among children might be due to immaturity of the immune system in these infection in these age group. Further studies are required to know the current burden in these population.

Conclusion

High prevalence of ESBL producing *E. coli* and *K. pneumoniae* was observed in our study.

E. coli is predominant ESBL producers than *K. pneumoniae*. Children under age five were found to be highly infected with urinary tract infection. *Escherichia coli and Klebsiella spp* are emerging highly as a multi-drug resistant. Imipenem, Tigecycline, Amikacin, Piperacillin-Tazobactam were found to be the most effective drug against the ESBL producing isolates. Nitrofurantoin and Gentamycin can be used as a drug of choice against non-ESBL producing isolates.

DECLARATION

Ethics approval and consent to participate

Ethical approval was taken from the Ethical Review committee of International Friendship Children's Hospital and Tri-Chandra Multiple Campus. Informed consent form was obtained from parents of the participants before their participation.

Competing interests

We have read Nepal journal of biotechnology policy on declaration of competing interest and declare that we have no competing interests.

Authors' contribution

Mr. Ujjwal Rimal, Mrs. Roshani Maharjan, and Dr. Shovana Thapa jointly performed the study.

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