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Microbiological and Clinical Profile of Uropathogenic *Escherichia coli* Isolates in Kathmandu University Hospital

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ABSTRACT

Background: Treatment of patients infected by multidrug resistant bacteria is a major challenge. Immunocompromised status, prolonged hospital stay, malignancy, diabetes are some of the risk factors for emergence of multidrug resistance. Our study focused on microbiological and clinical profile of multidrug resistant uropathogenic *Escherichia coli*.

Methods: This was a cross-sectional study conducted between June 2014-May 2015 in Kathmandu University Hospital. Urine sample from outpatients and inpatients from which *Escherichia coli* isolated was included. Specimen collection, culture, identification tests were done following guidelines given by American Society for Microbiology.

Results: Total number of urine samples received during the study were 3,554. *Escherichia coli* isolates were 645(18.14%) and 245(37.98%) were Extended Spectrum Beta-Lactamase producer. Extended Spectrum Beta-Lactamase producers were found more among inpatients 148(60.41%) [p<0.001], patients with underlying urological abnormalities 38 (15.51%) [p=0.0039], pregnant ladies 46(18.77%) [p=0.0028], diabetic patients 27 (11.02%) [p=0.0084], patients who received prior antibiotic therapy 155 (63.26%) [p=0.0043] than Extended Spectrum Beta-Lactamase non-producer. Malignancy was seen more among Extended Spectrum Beta-Lactamase producer having patients 5 (2.04%) [p=0.031] and all these isolates were more resistant to fluoroquinolones 168(68.57%), Trimethoprim-sulfamethoxazole 239 (97.55%) [p=0.0633], aminoglycosides [p=0.0001] but only 2(0.80%) were resistant to carbapenems.

Conclusions: Diabetes, pregnancy, malignancy, prior antibiotic therapy, underlying urological abnormalities were found associated with emergence of Extended Spectrum Beta-Lactamase producer in urine samples. Proper antibiotic usage may help to overcome the problem of emergence of antibiotic resistance.

Keywords: Extended Spectrum Beta-Lactamase, *Escherichia coli*, multidrug resistant.

INTRODUCTION

Antimicrobials remain the mainstay of empirical therapy; however, indiscriminate use of antibiotics in many developing countries including Nepal has resulted in the outbreak of drug resistant microorganisms.[1] It has been found and mentioned that, the importance of Extended Spectrum Beta-Lactamase producer (ESBL)-mediated infections has been increasing.[2-4] ESBL-producing infections have been found associated with both negative clinical outcomes and increased cost.[5,6]

Failure to adhere to proper infection control technique, unrationale use of antibiotics, unhygienic practices, increased uses of antibiotics in animal and plants and more so availability of antibiotics without prescription and counterfeit products of dubious quality in developing countries have resulted in spread of antimicrobial resistance and selection of multidrug resistant bacterial pathogens.[1,7,8] Unless we gather the information about the existing multidrug resistant (MDR) strains, the rate of emergence and spread of antimicrobial resistance cannot be reduced.[9]

With resistance to each additional class of antibiotics, ESBL-EK (*Escherichia, Klebsiella*) infections become a

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greater therapeutic challenge. Reliance on carbapenems has increased; because they are the only class of agents to which ESBL-EK remain almost uniformly susceptible. However, empirical treatment of suspected ESBL-EK infections with carbapenems has been associated with significant increases in carbapenem resistance in other organisms (e.g., *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus*).[10-12]

In 1983, the first outbreak involving extended spectrum beta-lactamase (ESBL) producing organisms was reported in Germany.[13] These ESBL-producing pathogens are now recognized globally as major causes of nosocomial and community-acquired infections.[14]

Several cases of multidrug resistant bacterial outbreaks of significant clinical concern have been frequently reported.[15-17]

Risk factors for colonization or infection with multidrug-resistant bacterial species include prolonged length of hospital stay, exposure to an ICU, receipt of mechanical ventilation, colonization pressure, and exposure to broad-spectrum antimicrobial agents, recent surgery, invasive procedures, and underlying severity of illness.[18,19]Patients who are infected with multidrug resistant bacteria have to stay in the hospital or have to be treated longer. Treating such patient has become a big challenge to the clinicians and patients for economic burden. Research on such situation may become a useful tool to overcome such situation in future. This study might be one of a good source to identify the burden of multidrug resistance pathogen, which might help to prepare guidelines for infection control and antibiotic policy.

METHODS

This was a cross-sectional study carried out at Kathmandu University Hospital, Dhulikhel, Nepal. Urine sample from outpatients and inpatients collected between June 2014 to May 2015 from which *Escherichia coli* was isolated (with special reference to ESBL) was included in the study. Clinical and epidemiological information was collected from the patient after taking informed consent from the patient.

Ethical clearance was taken from Institutional Review Committee of Kathmandu University Hospital before the study was conducted.

Specimen collection, culture, identification tests were done according to the guidelines given by American Society for Microbiology. [20] The antibiotic susceptibility test of the pathogens isolated from the clinical specimen against different antibiotics was done using Mueller Hinton agar (MHA) (Oxoid, United Kingdom) by the standard disk diffusion technique of modified Kirby-Bauer method as recommended by Clinical and Laboratory Standards Institute (CLSI). [21]

Definition of ESBL: They are capable of hydrolyzing penicillins, broad-spectrum cephalosporins and monobactams, but they do not affect the cephamycins or carbapenems and their activity is inhibited by clavulanic acid. [22]

The initial screen test for the production of ESBL was performed by using ceftriaxone (CRO) (30 µg), ceftazidime (CAZ) (30 µg) and cefotaxime (CTX) (30 µg) disks (Oxoid, UK). If the zone of inhibition (ZOI) was ≤ 25 mm for CRO, ≤ 22 mm for CAZ and/or ≤ 27 mm for CTX, the isolate was considered a potential ESBL- producer as recommended by CLSI.[21]

Combination disk (CD) method was used for the phenotypic confirmation of ESBL-producing strains in which CTX and CAZ (30 µg), alone and in combination with clavulanic acid (CA) (10 µg) was used (Becton Dickinson, USA). An increased ZOI of ≥ 5 mm for either antimicrobial agent tested in combination with CA versus its zone when tested alone confirmed ESBL.[21]

Data were analyzed by (SPSS) version 11.5 software and P value less than 0.05 was considered to be significant.

RESULTS

Total number of urine sample received during the study period was 3,554 out of which significant bacterial growth was observed in 835 (23.49%) samples and out of this total number *Escherichia coli* isolates was 645 (18.14%). Of total 645 *E. coli* 245(37.98%) were ESBL producer and 400(62.01%) were non-ESBL producer. Of total 245 ESBL *E. coli* 148 (60.41%) isolates were found among inpatients and 97 (39.59%) were found among outpatients. Of total 400 non-ESBL *E. coli* 142 (35.50%) isolates were found among inpatients and 258 (64.50%) were found among outpatients. ESBL *E. coli* isolates were found more among inpatients (60.41%) compared to Non-ESBL *E. coli* (35.5%), which was statistically significant. ($p<0.001$)

38 (15.51%) patients from whom ESBL *E. coli* was isolated had underlying urological abnormalities whereas only 32 (8.00%) of patients from whom non-ESBL *E. coli* was isolated had underlying urological abnormalities. ($p=0.0039$)

46 (18.77%) patients from whom ESBL *E. coli* was isolated were pregnant where as only 41 (10.25%) patients from whom non-ESBL *E. coli* was isolated were pregnant. (p value=0.0028)

91 (37.14%) patients from whom ESBL *E. coli* was isolated had complicated UTI whereas only 55 (13.75%) patients from whom non-ESBL *E. coli* was isolated had complicated UTI. (p<0.001)

27 (11.02%) patients from whom ESBL *E. coli* was isolated were diabetic whereas only 21(5.25%) patients from whom non-ESBL *E. coli* was isolated were diabetic. (p=0.0084)

5 (2.04%) patients from whom ESBL *E. coli* was isolated had malignancy whereas only 1 (0.25%) patients from whom non-ESBL *E. coli* was isolated had malignancy. (p value = 0.031)

155 (63.26%) patients from whom ESBL *E. coli* was isolated received prior antibiotic therapy whereas only

Table 1. Clinico-epidemiological characteristics of ESBL vs. non-ESBL *Escherichia coli* from urine samples

	ESBL isolates (n = 245, 38 %)	Non-ESBL isolates (n = 400, 62 %)	ESBL vs. Non-ESBL P value
Male patients	74 (30.20%)	124 (31.00%)	0.86
Female patients	171 (69.79%)	276 (69.00%)	
Outpatients	97 (39.59%)	258 (64.50%)	0.0001
Patients with underlying urological condition	38 (15.51%)	32 (8.00%)	0.0039
Pregnant patients	46 (18.77%)	41 (10.25%)	0.0028
Complicated UTI	91 (37.14%)	55 (13.75%)	0.0001
Uncomplicated UTI	154 (62.85%)	345 (86.25%)	
Patients with DM	27 (11.02%)	21 (5.25%)	0.0084
Patients with cancer	5 (2.04%)	1 (0.25%)	0. 031
Prior antibiotic therapy	155 (63.26%)	207 (51.75%)	0.0043

Table 2. Susceptibility profile of ESBL- *E. coli* and non-ESBL *E. coli* for various antibiotics.

Antimicrobial agents	ESBL-*E. coli* resistant (n=245)	Non-ESBL-*E. coli* resistant (n=400)	p-value
Ampicillin/Amoxicillin (10µg)	245 (100%)	267 (66.75%)	
Cefuroxime (30µg)	245 (100%)	233 (58.25%)	
Norfloxacin (10µg)	168 (68.57%)	245 (61.25%)	0.0633
Trimethoprim-sulfamethoxazole (1.25/23.75 µg)	239 (97.55%)	373 (93.25%)	0.0163
Gentamicin (10µg)	166 (67.75%)	102 (25.50%)	0.0001
Amikacin (30µg)	25 (10.20%)	5 (1.25%)	0.0001
Amoxycillin-clavulanic acid (20/10µg)	245 (100%)	15 (3.75%)	
Cefotaxime (30µg)	245 (100%)	12 (3.00%)	
Netilmicin (30µg)	148 (60.40%)	17 (4.25%)	0.0001
Aztreonam (30µg)	245 (100%)	7 (1.75%)	
Cefepime/Cefpirome (30µg)	211 (86.12%)	4 (1.00%)	
Cefoperazone-salbactum (75/30µg)	245 (100%)	2 (0.50%)	
Piperacillin-tazobactum (100/10µg)	245 (100%)	6 (1.5%)	
Ticarcillin-clavulanic acid (75/10µg)	245 (100%)	8 (2.00%)	
Imipenem (10µg)	2 (0.80%)	0 (0.00%)	
Meropenem (10µg)	2 (0.80%)	0 (0.00%)	
Includes intermediate and resistant isolates, based on CLSI criteria, 2013			

207 (51.75%) from whom non-ESBL *E. coli* was isolated received prior antibiotic therapy. (p value=0.0043).

Fluoroquinolone resistance was observed more among ESBL *E. coli* 168 (68.57%) than non-ESBL *E. coli* 245 (61.25%). [p=0.0633]

Trimethoprim-sulfamethoxazole resistance was observed more among ESBL *E. coli* 239 (97.55%) than non-ESBL *E. coli* 373 (93.25%). [p=0.0163]

Aminoglycoside resistance was also observed more among ESBL *E. coli* [p<0.001]

Of total 245 ESBL *E. coli* only 2 (0.80%) were resistant to Carbapenems.

DISCUSSION

Certain strains of *Escherichia coli* can cause a wide variety of intestinal and extra-intestinal diseases such as urinary tract infection, diarrhea, septicemia and neonatal meningitis.[23] Nosocomial infections caused by Extended Spectrum Beta-Lactamase producing pathogens are associated with risk factors such as elderly age, prolonged hospitalization, previous antibiotic use, and presence of invasive devices.[14,24] In our study duration total urine sample received was 3,554 out of which 835 had significant bacterial growth and out of this 645 (77.24%) were *Escherichia coli*. Among total 645 E. coli, which were screened for ESBL production, 245 (37.98%) were ESBL producer and 400 (62.02%) were non-ESBL producer. This finding was more than the finding done by Datta et.al in which out of total 140 strains of *E. coli*, which were screened for ESBL production, only 30 (21.4%) isolates were positive.[25] This may reflect that their antibiotic policy and antibiotic usage is better. ESBL *E. coli* isolates were found more among inpatients 148 (60.41%) compared to Non-ESBL *E. coli* 142 (35.50%), which was statistically significant (p<0.001). This means ESBL *E. coli* isolated were more encountered among hospital isolates indicating it might be nosocomial infection. This finding is similar to the finding observed by Husam. et.al in which ESBL producers among urinary *E. coli* isolates was significantly higher among in-patients. [26] Mean age of the patients with ESBL *E. coli* was 48.8 ± 22.3 and mean age of patients with non-ESBL *E. coli* was 40.8 ± 23.2. This means ESBL *E. coli* was detect more among patients of higher age group and old age remains one of the risk factor, which is similar to observation by Husam. et.al. [26] In our study of total 645 patients from whom *E. coli* was isolated 447 (69.30%) were female and 198 (30.70%) were male. It is true and it has been mention in many studies that females are more prone to urinary tract infection compared to the males. [27-29] Of total 245 patients from whom ESBL *E. coli* was isolated 171 (69.79%) were female and 74 (30.21%) were male.

In the past few years, the number of complicated UTI due to resistant gram-negative bacteria has risen, mainly due to spread of extended spectrum ß-lactamase (ESBL) bacteria, which pose a significant therapeutic challenge. Although a broad range of pathogens can cause complicated UTI, *Escherichia coli* remains the most common.[30]

Our study found similar observation as mentioned above in which 91 (37.14%) patients from whom ESBL *E. coli* was isolated had complicated UTI whereas only 55 (13.75%) patients from whom non-ESBL *E. coli* was isolated had complicated UTI (p<0.001) This means underlying urological abnormalities such as nephrolithiasis, benign prostatic hyperplasia, prostatic cancer, presence of invasive devices etc may be risk factor for acquiring urinary tract infection by ESBL *E. coli*.

In our study, 46 (18.77%) patients from whom ESBL *E. coli* was isolated were pregnant where as only 41 (10.25%) patients from whom non-ESBL *E. coli* was isolated were pregnant. (p value=0.0028) This means pregnancy might be risk factor for acquiring UTI by ESBL *E. coli*. In study conducted by Dutta et. al out of total 30 patients from whom ESBL *E. coli* isolates were detected, 11 (37%) were pregnant, which seems much more than our findings. [25] It may be because our maternity and birth center follows guidelines more strictly than their settings. In a study conducted by Aswani Srinivas et.al ESBL producing *E. coli* was significantly higher in diabetics (p value= 0.001) compared to non-diabetics. [31] In our study though we did not compare prevalence of ESBL *E. coli* among diabetics and non-diabetics we found higher number 27(11.02%) diabetic patients with ESBL *E. coli* in urine compared to less number 21(5.25%) diabetic patients with non-ESBL *E. coli*. (p value=0.0084) This result shows that diabetes may be another risk factor for acquiring multidrug resistant bacteria.

In our study 5 (2.04%) patients from whom ESBL *E. coli* was isolated had malignancy whereas only 1(0.25%) patients from whom non-ESBL *E. coli* was isolated has malignancy. (p value = 0.031) indicating underlying comorbid illness like malignancy might be a risk factor for acquiring ESBL *E. coli*.

In our study Fluoroquinolone resistance was observed more among ESBL *E. coli* 168 (68.57%) than non-ESBL *E. coli* 245 (61.25%). [p value=0.0633] which was quite

similar to finding observed by Husam S. et. al in which high levels of ciprofloxacin resistance was found among ESBL isolates. [26] This means fluoroquinolones should be used cautiously if ESBL *E. coli* is suspected from urine sample. In our study Trimethoprim-sulfamethoxazole resistance was observed more among ESBL *E. coli* 239 (97.55%) than non-ESBL *E. coli* 373 (93.25%). [p=0.0163] and aminoglycoside resistance was also observed more among ESBL *E. coli* [p<0.001]

In our study 155 (63.26%) patients from whom ESBL *E. coli* was isolated received prior antibiotic therapy whereas only 207 (51.75%) from whom non-ESBL *E. coli* was isolated received prior antibiotic therapy. (p value=0.0043) This finding might suggest that prior antibiotic therapy might be a risk factor for acquiring ESBL *E. coli*.

This study do has some limitations. Since, it was carried out in a single center the results may not be applicable to settings with a different epidemiology. In addition, only urine sample was screened for ESBL *E. coli* not including other clinical sample like blood, pus, wound swab, sputum etc.

CONCLUSIONS

Several factors were found associated with emergence of Extended Spectrum Beta-Lactamase producing *Escherichia coli* from urine sample. Extended Spectrum Beta-lactamase producing *Escherichia coli* was found more resistant to other group of drugs as well. But less than one percent of them were resistant to carbapenem. Hospital-acquired Extended Spectrum Beta-Lactamase producers are emerging challenge and proper antibiotic usage following antibiotic policy may help to some extent to over come this.

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