

PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA* SPP. FROM URINARY SPECIMEN IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Escherichia coli and *Klebsiella* spp. are two important uropathogens showing resistance to beta-lactams by producing extended spectrum beta lactamase (ESBL), leading to difficulty in treating infections with these bacteria. Majority of the ESBLs are of TEM, CTXM, and SHV types reported from Nepal. This study was conducted in Clinical Microbiology Department of Nepal Medical College Teaching Hospital from November 2021 to mid-January 2022 to determine prevalence of ESBL and antimicrobial susceptibility pattern among two common uropathogens. A total of 402 *E. coli* and *Klebsiella* spp. isolated from urine samples were included in the study. ESBL producing strains were identified by combination disc method and antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method. PCR was done to detect the TEM and CTXM genes. Prevalence of ESBL among *E. coli* and *Klebsiella* spp. was found to be 14.9% (60/402). Among the *E. coli*, 16.1% (58/360) and only 4.8% (2/42) of *Klebsiella* spp. were ESBL producers. Antibiogram showed, 56.7% ESBL producers were susceptible to ciprofloxacin, 46.7% to co-trimoxazole, 96.7% to nitrofurantoin. However, all isolates were susceptible to amikacin, piperacillin-tazobactam, and carbapenems (both meropenem and imipenem). Among total 30 ESBL isolates selected by systematic random sampling, 3 isolates (all three *E. coli*) showed presence of TEM and 3 other isolates (two *E. coli* and one *Klebsiella* spp.) showed CTXM genes.

KEYWORDS

Prevalence, ESBL, *E. coli*, *Klebsiella*, TEM, CTXM, urine

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INTRODUCTION

Antimicrobial resistance (AMR) is a global health challenge nowadays¹ and urinary tract infection (UTI) is one of the common clinical problem.²⁻⁴ *E. coli* and *Klebsiella* spp. are two most common Gram negative urinary pathogens and drug resistance encountered in these bacteria has lead to difficulty in empirical treatment.⁵⁻⁷ Resistance to beta- lactams by production of extended spectrum beta lactamase (ESBL) enzyme is most frequently encountered problem as all ESBLs are resistant to all beta lactams except carbapenems, cephamycins and clavulanic acid.^{8,9} They spread quickly to other genus and species through horizontal gene transfer. They belong to Ambler's class 'A' beta-lactamases.¹⁰ They have various genotypes, the most common being TEM, CTXM and SHV.¹¹

ESBL producing *E. coli* and *Klebsiella* spp. are clinically important as they are multi drug resistant (MDR) with few treatment options and mortality rate due to undiagnosed ESBL has been found in the range from 40-100%.¹²⁻¹⁵ Carbapenem resistance also has been reported among ESBL producers greatly shrinking the treatment options.¹¹

The prevalence of ESBL among various bacteria in any given time varies according to the health care set-up.¹ It has also been shown that the prevalence of ESBL infection is higher in resource poor developing countries.¹⁶⁻¹⁸

This study was designed to determine the prevalence of ESBL and antimicrobial susceptibility pattern among *E. coli* and *Kiebsiella* spp. isolated from urine samples in a tertiary care hospital along with detection of TEM and CTXM.

MATERIALS AND METHODS

A total of 2,610 urine samples received for bacterial culture and sensitivity in Clinical Microbiology Laboratory of Nepal Medical College Teaching Hospital (NMCTH) from November 2021 to mid-January 2022 were included in the study after obtaining ethical approval from Nepal Medical College Research and Ethical Committee. *E. coli* and *Klebsiella* spp. isolated from urine samples were identified based on colony morphology, Gram stain, and biochemical tests by using standard Microbiological techniques.¹⁹ Antimicrobial susceptibility test was done in Mueller Hinton Agar (Hi-Media) by Kirby Bauer's disc diffusion method as per CLSI.²⁰ Isolates that were resistant to at least one each from three different classes

of antimicrobial agents were regarded as MDR. ESBL producing *E. coli* and *Klebsiella* spp. were phenotypically confirmed by combination disc method.

Phenotypic confirmatory combination disc method for detection of ESBL producers

E. coli and *Klebsiella* spp. were confirmed for ESBL production by phenotypic test. In brief, lawn culture of isolated bacteria was made on Mueller Hinton Agar (MHA) and ceftazidime (30µg) and cefotaxime (30µg) alone and in combination with clavulanic acid (10µg) were placed 25 mm apart (centre to centre) and incubated aerobically at 37°C overnight. An increase of zone of inhibition 5 mm or more for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone were confirmed as ESBL producing strains as per CLSI.²⁰

E. coli ATCC 25922 (ESBL non producer) and *Klebsiella pneumoniae* ATCC 700603 (ESBL producer) were used as controls.

Method for gene detection

DNA extraction was done from 30 ESBL isolates selected by systematic random sampling technique among total 60 ESBL producers using Qiagen kit (manufactured by QIAquick, India) according to standard guideline.²¹ It was then subjected for Polymerase Chain Reaction (using conventional thermocycler) which includes denaturation, annealing and extension under special conditions for detection of common type of TEM and CTXM genes.²² Primers used were:^{23,24}

TEM (296 bp):

Forward: TCCGCTCATGAGACAATAACC

Reverse: ATAATACCGCACCACATAGCAG

CTXM (560 bp):

Forward: TTGCGATGTGCGAGTACCAGTAA

Reverse: CTCCGCTGCCGGTTTTATC

PCR product was then run in gel electrophoresis and bands formed were read by Gel Documentation System for detection of genes responsible for ESBL.²⁵ All obtained data were analysed using MS-Excel 2007.

RESULTS

Among total 2,610 urine samples processed, 448 (17.2%) yielded significant growth. Out of these, 430 (95.9%) were Gram negative bacilli (GNB) and 18 (4.1%) were Gram positive cocci (GPC) (Table 1). Out of all (448) urinary isolates, *E. coli* were 360 (80.3%) and *Klebsiella* spp. were

Table 1: Distribution of organisms among culture positive urine samples

Organism	n	%
<i>E. coli</i>	360	80.3%
<i>Klebsiella</i> spp.	42	9.4%
<i>Proteus</i> spp.	4	0.8%
<i>Pseudomonas</i> spp.	18	4.2%
<i>Citrobacter freundii</i>	6	1.3%
<i>Staph. saprophyticus</i>	10	2.2%
<i>Enterococcus</i> spp.	8	1.8%
Total	448	100%

Table 2: Age wise distribution of ESBL.

Age group (years)	n (%)
<20	12 (20%)
21-40	24 (40%)
41-60	20 (33.3%)
>60	4 (6.7%)
Total	60 (100%)

42 (9.4%). Prevalance of ESBL among these *E. coli* and *Klebsiella* spp. was found to be 14.9% (60/402); ESBL *E. coli* were 16.1%% (58/360) and ESBL *Klebsiella* spp. were 4.8% (2/42). All ESBL producers were isolated from outpatients. Age wise, more ESBL prevalence was seen among reproductive age group of 21-40 years (24/60), followed by 41-60 years (20/60), <20 years (12/60) and then >60 years (4/60) (Table 2).

Table 3: Antibiotic susceptibility pattern of ESBL producing *E. coli* and *Klebsiella* spp.

Name of antibiotics	Susceptibility/60 (%)
ciprofloxacin	34 (56.7%)
ofloxacin	35 (58.3%)
co-trimoxazole	28 (46.7%)
amikacin	60 (100%)
nitrofurantoin	58(96.7%)
piperacillin-tazobactam	60 (100%)
meropenem	60 (100%)
imipenem	60 (100%)

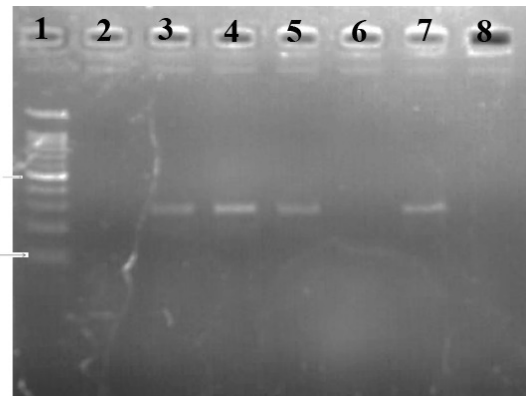


Fig. 1: TEM genes. (TEM-(296bp): lane 1 is Molecular marker, lane 2, 3, 4 are samples, lane 5 is positive control, lane 6 is negative control, and lane 7, 8 are samples).

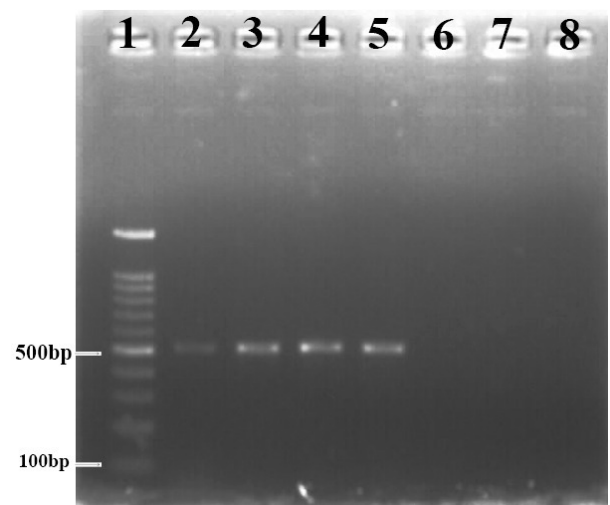


Fig. 2: CTXM genes. (CTXM-(560bp): lane 1 is molecular marker, lane 2, 3, 4 are samples, lane 5 is positive control, lane 6 is Negative control, and lane 7, 8 are samples).

Antibiogram of ESBL producing organisms show that 56.7% ESBL producers were susceptible to ciprofloxacin, 46.7% to cotrimoxazole, 96.7% to nitrofurantoin. All ESBL isolates were susceptible to amikacin, piperacillin-tazobactam, meropenem, imipenem found by phenotypic Kirby Bauer's disc diffusion method (Table 3). All ESBL isolates were susceptible to carbapenems (both meropenem and imipenem).

An isolate that is resistant to at least one antibiotic from three or more drug classes is defined as MDR. MDR among ESBL producers was found to be 83.3% (50/60). All (2/2) of ESBL producing *Klebsiella* spp. and 82.7% (48/58) ESBL producing *E. coli* were MDR.

Among ESBL producers, TEM and CTXM genes were looked for. Out of 60 phenotypically

confirmed ESBL producers, 30 isolates selected by systematic random sampling were subjected for detection of TEM and CTXM by PCR. 3 isolates (all 3 *E. coli*) showed presence of TEM (Fig. 1) and 3 other isolates (2 *E. coli* and 1 *Klebsiella* spp.) showed CTXM genes (Fig. 2).

DISCUSSION

UTI is the second most commonly diagnosed infectious illness worldwide, with about 150 million diagnosed yearly. Gram-negative bacilli are the most common uropathogens with *E. coli* being the most prevalent type accounting for 75-90%.²⁶ Extended-spectrum beta-lactam antibiotics have widely been used for treatment of serious Gram-negative infections. However, bacterial resistance has emerged among these isolates due to production of extended-spectrum beta-lactamases (ESBLs).²⁷ The increasing rate of resistance to the more commonly used antibiotics has made empirical treatment more difficult. UTI complicated by ESBL organisms tend to lead to uncertain outcomes and prolong hospitalization, due to multi-drug resistance.²⁸

This study was done with an objective of determining the prevalence of ESBL among urinary isolates of *E. coli* and *Klebsiella* spp. In this study, *E. coli* was the most common urinary isolate followed by *Klebsiella* spp. Both constituted 89.7% of the total urinary isolates and prevalence of ESBL was 14.9% (ESBL *E. coli* being 16.1% and ESBL *Klebsiella* spp. being 4.8%). This finding is similar to findings of other studies from Nepal conducted during different time periods.^{1,18,29} Similar type of sample included in the study (as all studies done were from urine samples) and similar study set up in all hospitals of Nepal might be the reason for similar findings in these studies. However, few other studies done in China, Italy, Tanzania have shown very high prevalence of ESBL as compared to our study.³⁰⁻³² Differences in the specimen types included in the study and different hospital set ups could be the reasons for the variations.

ESBL prevalence is commonly high among inpatients compared to outpatients according to previous literatures.¹⁻⁵ However, we found contrasting result that it was more common among outpatients in our study. This might be due to very less number of admitted patients (only 6% of total patients) in hospital during this study period of COVID pandemic as most patients has consulted in OPD only without getting admitted in hospital except emergency medical condition. Other reason for this could

be due to carriage of ESBL among outpatients due to use of antibiotics in animal farming.

UTI is more common among females and specially among reproductive age group.^{6,7} The prevalence of ESBL producing *E. coli* and *Klebsiella* spp. reflected this trend with the most common age group 21-40 years followed by 41-60 years in our study and also from studies done elsewhere.^{33,34}

ESBL producers in this study showed 56.7% susceptibility to ciprofloxacin, 58.4% to ofloxacin, and 46.7% to cotrimoxazole. Similar result has been found in a study done in Tanzania.³⁵ The resistance shown by isolates may be due to widespread use of these drugs empirically because of being relatively cheap and easy to administer.^{36,37}

In our study, we found all isolates were susceptible to amikacin among ESBL producing *E. coli* and *Klebsiella* spp. However, similar study reported from Kathmandu Medical College Teaching Hospital showed 9.5% resistance to this antibiotic among the ESBL producing *E. coli* and *Klebsiella* spp.¹ 26.0% uropathogenic *E. coli* and *Klebsiella* spp. in another study in Chitwan were resistant to amikacin irrespective of their ESBL status.²⁹ Many similar and few contrasting results have been reported in various research studies from various places.³⁰⁻³⁴ Different antibiotic policies in various hospitals and places might be the reason for such findings. A study has shown that combination therapy of amikacin with tazobactam has good effect for treating ESBL producing organisms.³⁵ Our finding shows aminoglycoside have good activity against clinically important GNB and susceptibility to piperacillin tazobactam was seen among all isolates. In addition, aminoglycoside is not firstline drug used routinely which might be the reason for not developing resistance by organisms to these antibiotics.

Nitrofurantoin is one of the useful antibiotic against ESBL producing Gram negative uropathogens.¹ We also found similar report that 96.7% of the isolates were susceptible to it. Many research findings are similar to our findings (showing 94%-100% susceptibility, however, few results are in contrast also.^{23,24,29-34} There is no research finding showing high rate of resistance to nitrofurantoin by uropathogens making this drug as suitable option for treatment of *E. coli* and *Klebsiella* spp. in our set-up.

Resistance to carbapenems occurs due to production of carbapenemase enzyme by these

isolates or due to efflux pump mechanism.¹ All ESBL producing *E. coli* and *Klebsiella* spp. in this study were susceptible to carbapenems (both meropenem and imipenem). We found similar reports in other studies by Chander *et al* (2013) and Mahaseth *et al* (2019) too.^{1,29}

An isolate that is resistant to at least one antibiotic from three or more drug classes which are structurally different and have different molecular targets is defined as MDR.¹ There is strong correlation between ESBLs and MDR.²⁴ In our study, 83.3% of ESBL producing isolates were multidrug resistant. We found our result matching with findings of other studies also in which prevalence of MDR was found to be 86.7% and 89.3% respectively.^{1,29} This should alarm us against this global challenge of antimicrobial resistance.

Mostly, TEM1 and CTXM1 along with few SHV genes have been reported from ESBL isolates.^{5,9,10,36} We found three TEM (all 3 from *E. coli*) and other three CTXM genes (2 from *E. coli* and 1 from *Klebsiella* spp.) among thirty phenotypically confirmed ESBL producing *E. coli* and *Klebsiella* spp. selected by systematic random sampling technique. Our finding

is similar to that of other studies in various places.^{32,37-38} However, we didn't plan for detection of SHV gene and other TEM and CTXM types which is the limitation of our study.³⁹ Further, we detected TEM1 and CTXM1 (common types) which might not be present in all cases among ESBL producers.³⁹⁻⁴¹

In conclusion, prevalence of ESBL producing *E. coli* and *Klebsiella* spp. isolated from urine specimen was found to be high 3 out of 30 (10%) was due to TEM and another 10% was due to CTXM genes. All ESBL producing isolates originated from outpatients which is still alarming since it indirectly points towards a high prevalence of ESBL in the community.

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