# Extended Spectrum Beta-Lactamase Producing Gram Negative Pathogens in Urine Samples of Patients Visiting B.P. Koirala Memorial Cancer Hospital, Bharatpur, Nepal

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## ABSTRACT

**Objectives:** To isolate and identify extended spectrum beta-lactamases producing Gram negative bacteria in urine samples of patient visiting B.P. Koirala Memorial Cancer Hospital, Chitwan, Nepal.

**Methods:** Urine samples were processed to isolate and identify Gram negative bacteria by different biochemical tests (Gram stain, catalase test, oxidase test, indole test, MR test, VP test, citrate utilization test, TSIA test), antimicrobial susceptibility test was done as recommended by Clinical Laboratory Standard Institute (CLSI) and production of ESBL was determined by combination disk diffusion method.

**Results:** Out of total 142 samples, prevalance of UTI in cancer and cancer suspected patients was found to be 24.64%. Of the total Gam negative isolates, the most predominant organism was found to be *E. coli* 63% followed by *Klebsiella* 26% and others 11.11%. Among 27 Gram negative isolates, MDR was found to be 81.48%. The prevalence of ESBL producers among the total Gram negative isolates was 11.11%. The higher rate of growth was seen in age group of 60- 70 (29.62%). Higher prevalence of bacterial growth was observed in male. Polymyxin B, Gentamycin and Nitrofurantoin were the most effective antibiotics towards Gram negative bacteria.

**Conclusion:** The prevalence of MDR and ESBL among Gram negative uropathogens isolated from cancer and cancer related patients are quite high. Therefore, it is essential to have a regular monitoring of ESBL producing clinical isolates in laboratory practice.

**Keywords:** Gram negative bacteria, antimicrobial resistance, MDR, ESBL, UTI.

## INTRODUCTION

Urinary tract infections (UTIs) are infections that occur in the urinary system due to the presence, growth, and spread of bacteria. These infections typically begin when bacteria from the digestive tract invade the urethra, leading to multiplication and infection. In underdeveloped countries, UTIs are among the most common bacterial infections encountered in clinical settings (Mandracchia, 2000). While bacteria are the primary cause, UTIs can also result from fungi or parasites. Most uropathogens belong to the Enterobacteriaceae family (Roy et al., 2016). UTIs are prevalent, affecting both hospitalized patients and individuals in the community. The bacteria responsible for UTIs possess specific traits, such as toxin production, the ability to adhere to surfaces, and the release of siderophores, which enable them to invade the urinary tract and spread infections among different individuals (Luthje and Brauner, 2014).

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Evidence indicates that cancer patients have a high prevalence of antimicrobial resistance (Shrestha et al., 2021). Their compromised immune systems increase the risk of severe opportunistic infections (Rolston and Bodey, 2009). Cancer patients are particularly susceptible to UTIs due to prolonged immunosuppression, complex treatments, and catheter use (Tigabu et al., 2020). Treating UTIs in these patients is challenging because many are at a higher risk for antimicrobial resistance (AMR) due to long-term chemotherapy and frequent antibiotic use (Parikh and Bhat, 2015).

Extended-spectrum beta-lactamase (ESBL)- producing Gram-negative bacteria are a specific group of bacteria that produce the enzyme β-lactamase, which can inactivate a wide range of beta-lactam antibiotics, including penicillins and cephalosporins. Consequently, infections caused by these bacteria, including UTIs, are difficult to treat. ESBL enzymes have been identified in various Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella spp*., as well as in non-lactose fermenters like *Pseudomonas aeruginosa* (Smet et al., 2018). Persistent exposure to beta-lactam antibiotics has led to ongoing production and mutation of β-lactamase in these bacteria, allowing them to resist even newly developed beta-lactam antibiotics (Paterson and Bonomo, 2005).

Antimicrobial resistance has become a major global healthcare concern, particularly regarding infections caused by multidrug- resistant (MDR) microorganisms, which impose significant clinical and economic burdens (Kadri et al., 2021). While many of these infections are often linked to healthcare settings, there is an increasing prevalence of MDR bacteria causing community-acquired infections (Bader et al., 2017).

The detection of ESBL-producing bacteria in samples like urine is important as it acts as an epidemiological marker of colonization, indicating the potential for these organisms to be transmitted to other patients (Paterson and Bonomo, 2005). In Nepal, research on urinary tract infections (UTIs) among cancer patients is limited. This study aims to improve the treatment of cancer patients suffering from UTIs by providing insights into the antibiotic susceptibility profiles and identifying drug-resistant strains. Additionally, it will gather data on the age groups and genders most affected by these infections.

## METHODS

A non-purposive cross-sectional study was conducted at the microbiology laboratory of B.P. Koirala Memorial Cancer Hospital (BPKMCH) from January to April 2023. The study included 142 urine samples from patients of all ages and sexes suspected of urinary tract infections (UTIs), collected aseptically in clean, leak-proof containers by the trained technicians following standard guidelines (Cheesbrough, 2006). Samples were labeled correctly and accompanied by patient histories, including age, sex, and symptoms. Only samples collected on the same day as processing and without contamination were included; those with improper labeling or insufficient volume were excluded. The specimens were processed promptly under aseptic conditions.

Urine samples were inoculated on CLED media and incubated at 37°C for 24 hours. The identification of various Gram-negative bacterial isolates from positive culture plates was carried out using standard microbiological techniques, which included examining colony morphology, performing Gram staining, and assessing various biochemical properties (Cheesbrough 2006). The Gram-negative bacterial isolates were identified based on their reactions in a series of biochemical tests such as catalase, oxidase, indole, methyl red (MR), Voges-Proskauer (VP), citrate utilization, triple sugar iron (TSI), motility, gas production, hydrogen sulfide production, and urease tests. Pure colonies from the media plates were further inoculated on different biochemical media, and the test results were recorded.

Susceptibility testing of various bacterial isolates against different antibiotics was conducted using the modified Kirby-Bauer disk diffusion technique on MHA media, following Clinical Laboratory Standard Institute (CLSI) guidelines. Isolates that were non-susceptible to at least one agent in three or more antimicrobial categories were classified as MDR (Magiorakos et al., 2012). Additionally, isolates were screened for potential ESBL production using Ceftazidime (CAZ) (30 µg) and Cefotaxime (30 µg), with zone sizes of ≤22 mm for Ceftazidime and ≤27 mm for Cefotaxime indicating probable ESBL producers. Suspected ESBL-producing strains were then subjected to phenotypic confirmatory testing according to CLSI guidelines (CLSI, 2016).

The possible ESBL producer were tested for confirmation by using combination disk method, using Ceftazidime (30µg) and Ceftazidime + Clavulanic acid (CAC) (30/10 µg), Cefotaxime (CTX) (30 µg), Cefotaxime + Clavulanic acid (CEC) (30/10 µg). An increase in the zone diameter by <5 mm around the disks containing Cephalosporin with Clavulanic acid over the disks containing Cephalosporin alone indicated ESBL production (CLSI 2016).

## RESULTS

A total of 142 non repetitive urine samples from both inpatients and outpatients were investigated during a four months' study period. The prevalence of UTIs in cancer or cancer suspected patients was found to be 24.6% (35/142), whereas prevalence of Gram negative pathogens

was found to be 19.0% (27/142).

Among the 27 samples that exhibited growth of gram-negative bacteria, *Escherichia coli* emerged as the most prevalent pathogen, accounting for 62.9% (17/27). This was followed by *Klebsiella species* with 25.9% (7/27) of the samples, *Pseudomonas* and *Proteus* were less common, comprising 7.4% (2/27) and 3.7% (1/27) of samples, respectively.

Maximum number of Gram negative uropathogens was obtained from male 25% (14/50) and in age group 60-70 years 28.6% (8/27). The association between gender and growth of bacteria was found statistically insignificant ($p>0.05$) where significant between age group of patients and bacterial growth ($p<0.05$).

All Gram negative uropathogens were found to be sensitive to Polymyxin-B followed by Gentamycin 51.8%, Nitrofurantoin 48.1%, while resistant with Ampicillin 88.8% followed by Cefotaxime and Cefepime 81.5% (Table 3). Among the Gram negative uropathogens containing samples, MDR were found to be 81.48% (22/27). All *Peudomonas* spp. (100%) were found to be MDR followed by *Klebsiella* (85.7%) and *E. coli* (82.3%) (Table 4).

Of the total 27 isolates, 20 were suspected of being ESBL producers. Among the 20 screen positive samples, confirmed ESBL producers were 11.11% (3/20). From the total ESBL producers, *E. coli* was the most predominant isolate with 66.67%(2) followed by *Klebsiella* 33.33% (Table 5).

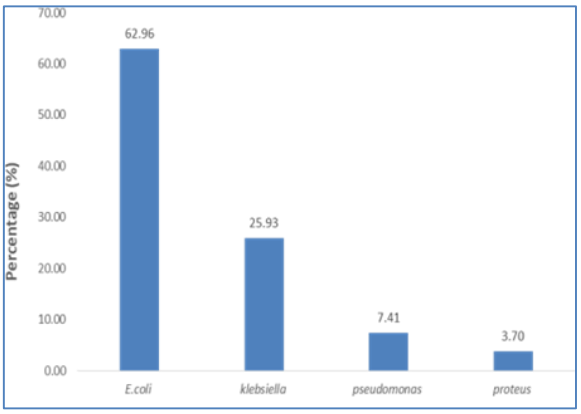


**Figure 1: Distribution of Gram negative isolates from urine sample**

Highest number of ESBL producers was obtained from the patients with age group of 30-40, 50-60 and 70-80 that is 33.33% and according to gender wise distribution of ESBL producer, maximum number of ESBL isolates were found from male patients 66.7%. There was no significant association between ESBL producers and age group as well as gender of patients ($p>0.05$) (Table 6).

**Table 1: Gender wise distribution of UTIs**

| Gender value | Growth n (%) | No growth n (%) | Total | p-value |
|---|---|---|---|---|
| Female | 13(15.11%) | (87.8%) | 86 | 0.14 |
| Male | 14(25%) | 42(75%) | 50 | |
| **Total** | **27** | **115** | **142** | |

**Table 2: Growth on the basis of age group**

| Age Group | No growth n (%) | Growth n (%) | Total | p- value |
|---|---|---|---|---|
| 0-10 | 2(1.7) | 2(7.40) | 4 | <0.01 |
| 10-20 | 6(5.2) | 0(0.1) | 6 | |
| 20-30 | 11(9.5) | 2(7.40) | 13 | |
| 30-40 | 23(20) | 4(14.81) | 27 | |
| 40-50 | 10(15.65) | 3(11.11) | 21 | |
| 50-60 | 26(22.60) | 3(11.11) | 29 | |
| 60-70 | 17(14.70) | 8(28.62) | 25 | |
| 70-80 | 12(10.43) | 5(18.51) | 17 | |

**Table 3: Antibiotic susceptibility pattern of growth negative isolates**

| Antibiotics | Sensitive n (%) | Resistance n (%) |
|---|---|---|
| Ampicillin | 3 (11.11) | 24 (88.86) |
| Gentamycin | 14 (51.85) | 13 (48.15) |
| Ceftazidime | 6 (22.22) | 21 (77.77) |
| Cefotaxime | 5 (18.52) | 22 (81.48) |
| Cefepime | 5 (18.52) | 22 (81.48) |
| Cotrimoxazole | 6 (22.22) | 21 (77.77) |
| Nitrofurantoin | 13 (48.15) | 14 (51.85) |
| Polymyxin-B | 27 (100) | 0 (0) |
| Levofloaxacin | 7 (25.93) | 20 (70.04) |

**Table 4: Distribution of MDR isolates**

| Organism | MDR n (/%) | Non-MDR n (%) | Total |
|---|---|---|---|
| *E. coli* | 14(82.35) | 3(17.65) | 17 |
| *Klebsiella spp* | 6(85.71) | 1(14.29) | 7 |
| *Pseudomonas spp* | 2(100) | 0 | 2 |
| *Proteus spp* | 0 | 1(100) | 1 |
| **Total** | **22** | **5** | **27** |

**Table 5: ESBL production profile of Gram negative isolates**

| Organism | Total-isolates | No of suspected ESBL producers n (%) | Confirmed ESBL producers n (%) |
|---|---|---|---|
| *E. coli* | 17 | 13(65%) | 2(66.66%) |
| *Klebsiella spp* | 7 | 6(30%) | 1(33.33%) |
| *Pseudomonas spp* | 2 | 1(5%) | 0 |
| *Proteus spp* | 1 | 0 | 0 |
| **Total** | **27** | **20 (74.07%)** | **3 (11.11%)** |

**Table 6: Age and gender wise distribution of ESBL producers**

| Category | ESBL producers n (%) | Negative n (%) | Total | p-value |
|---|---|---|---|---|
| **Age group** | | | | |
| 0-10 | 0 | 2(8.33) | 2 | 0.185 |
| 10-20 | 0 | 0 | 0 | |
| 20-30 | 0 | 2(8.33) | 2 | |

| | | | | |
|---|---|---|---|---|
| 30-40 | 1(33.33) | 3(12.50) | 4 | |
| 40-50 | 0 | 3(12.50) | 3 | |
| 50-60 | 1(33.33) | 2(8.33) | 3 | |
| 60-70 | 0 | 8(33.33) | 8 | |
| 70-80 | 1(33.33) | 4(16.67) | 5 | |
| **Gender** | | | | |
| Female | 1(33.33) | 12(50) | 13 | |
| Male | 2(66.67) | 12(50) | 14 | 0.585 |
| **Total** | **3** | **24** | **27** | |

## DISCUSSION

Urinary tract infections (UTIs) caused by ESBL- producing bacteria are increasingly common in both hospitalized and outpatient settings. The rise in drug resistance among these organisms has complicated UTI treatment, often requiring the use of more expensive broad-spectrum antibiotics, such as third-generation cephalosporins. The challenges in detecting ESBLs through standard antimicrobial susceptibility assays and delays in reporting ESBL production by gram-negative bacilli contribute to increased morbidity, mortality, and healthcare costs (Mehrgan et al., 2008). The growing concern over drug resistance in Enterobacteriaceae is exacerbated by the microorganism's genetic adaptability, the selective pressure of antibiotic use, and global population movement, leading to the emergence and spread of resistant bacterial strains worldwide (Svard, 2007).

The goal of this study was to identify MDR isolates and ESBL producers in Gram negative pathogen in urine sample from patient visiting BPKMCH. A total 142 urine specimens were taken as a sample among them 35 (24.64%) showed significant growth. Similarly, previous studies had also reported low growth rate of 25.5% (Awasthi et al., 2015). Similarly, study done by Shrestha et al. (2021) reported (24%) of significant bacteriuria, which is similar to our study. Our finding was lower than those reported by Gupta et al. (2022) who reported 40.7% bacterial growth.the possible cause of low growth of positivity is that the sample might be from patients under treatment, infections due to slow growing organisms or due to those organisms that were not able to grow on the routine media that were not Used (Karki, 2004).

Among the 35 bacterial isolate 77.14%(27/35) were Gramnegative and 22.85%(8/35) were Gram positive. The percent of Gram-negative bacterial isolates in this study was comparable to the study done by Adikari et al (2019) and singh et al (2020) in Nepal who showed 72.9% and 82.6% Gramnegative isolate. Similarly, Pandey et al. (2020) in Nepal found 91.40% of isolates were Gram-negative.

In this study, the prevalence of UTIs was higher in males (25.45%) compared to females (16.46%), aligning with other research by Martin et al. (2016), which reported rates of 4.8% in males and 3.0% in females. Similarly, Shashwati et al. (2014) found higher growth rates in males (52.54%) than in females (52.28%). However, Rimal et al. (2017) observed a higher prevalence in females (27.45%) compared to males (16%). The increased growth rate in males may be due to direct contact with infected bodily fluids (Dhoubhadel et al., 2002).

In this study, the highest bacterial growth was observed in patients aged 50-60 years (20.14%), followed by those aged 30-40 years (18.66%), with the lowest growth in the 0-10 age group (2.24%). This finding aligns with Rahim et al. (2018), who also reported the highest growth in 50-60 age group (27.4%) and the lowest in 10-20 age group (3%). Among the 27 gram-negative isolates, *E. coli* was the most predominant pathogen (63%), followed by *Klebsiella* (26%), *Pseudomonas* (7.4%), and *Proteus* (3.7%). This predominance of *E. coli* is consistent with findings by Fenta et al. (2020), who reported *E. coli* as the leading urinary pathogen (63.63%), and studies in Lebanon by Daoud et al.

(2015) showing *E. coli* incidence rates between 60.53% and 73.98%. Other studies, such as Hayajneh et al. (2015), also reported *E. coli* as the most frequent UTI isolate (70%), followed by *Klebsiella* (14%) and *Pseudomonas* (5%). However, our findings differ from Aboderin et al. (2009), which identified *Pseudomonas aeruginosa* as the predominant bacterium. The high prevalence of *E. coli* observed in this study is also consistent with research by Pandit et al. (2020), Yadav et al. (2015), and Baral et al. (2012) in Nepal.

In this study, all isolated gram-negative bacteria were tested with specific antibiotics according to CLSI guidelines (2016). Among the nine antibiotics used, Polymyxin B was the most effective, with 100% sensitivity, followed by Gentamycin (51.85%), Nitrofurantoin (48.15%), Levofloxacin (25.93%), Ceftazidime (22.22%), Cotrimoxazole (22.22%), Cefotaxime (18.52%), Cefepime (18.52%), and Ampicillin (11.11%). Most isolates were sensitive to Polymyxin B and resistant to Ampicillin (88.88%), consistent with findings by Joshi et al. (2018), who reported 100% sensitivity to Polymyxin B. Bukhari et al. (2019) also supported these results, showing Polymyxin B as the most effective drug against uropathogens, followed by aminoglycosides, similar to our findings. Metri et al. (2011) found 67.6% sensitivity to Gentamycin, with high resistance to Ampicillin (94.5%).

MDR is defined as resistance to at least one antibiotic from three or more structurally different drug classes with distinct molecular targets (Chander and Shrestha, 2013). In our study, 81.48% of gram-negative isolates were MDR. This is similar to Panta's (2013) study at the National Kidney Centre, which reported 85.83% MDR among 19.92% growth. However, other studies showed lower MDR rates, such as 55.9% (Poudyal et al., 2011), 64% (Shakya et al., 2017), and 64.9% (Parajuli et al., 2017). In underdeveloped countries like Nepal, self- medication, expired or counterfeit drugs, and inadequate hospital controls contribute to rising antibiotic resistance (Thakur et al., 2013).

In this study, 82.35% of *E. coli,* 85.71% of *Klebsiella*, and 100% of *Pseudomonas* isolates were MDR, while *Proteus* was non-MDR. These findings are similar to Ghimire et al., who reported MDR prevalence in E. coli (75%) and *Klebsiella* (94.4%).

In our study, we screened 27 MDR gram- negative bacteria for ESBL production, with 20 isolates initially testing positive. Of these, 3 were confirmed as ESBL producers, resulting in a prevalence rate of 11.11% among the total gram-negative isolates. This prevalence is lower compared to other studies, such as Chander and Shrestha (2013), which reported a 13.5% prevalence, and Guragain et al. (2019), which found a prevalence of 29.8%. Additional studies have reported even higher rates: 24% (Ansari et al., 2015), 25% (Khanal et al., 2013), 25.8% (Neupane et al., 2016), 26.8% (Pant et al., 2016), and 33.2% (Pokharel et al., 2014). The lower prevalence observed in our study may be due to factors such as limited sample collection and lower patient flow in the hospital (Walson et al., 2001).

In this study, the occurrence of ESBL-producing uropathogens was predominantly *E. coli* (66.66%) and *Klebsiella* spp (33.33%). These findings align with prior studies, where *E. coli* prevalence ranged from 45.2% to 67.2% (Mehrgan&Rahbar, 2008) and *K. pneumoniae* from 44.4% to 52% (Feizabadi et al., 2006). Supriya et al. (2004) reported *E. coli* at 49.8%,

*K. pneumoniae* at 37.8%, and *P. aeruginosa* at 6.5%. Our results showed a higher prevalence of ESBL producers in males (66.67%) compared to females (33.33%), which contrasts with Nipa et al. (2016), who observed a higher prevalence in females (62.4%) than in males (59.3%). Additionally, Rimal et al. (2018) found a greater prevalence of ESBL isolates in females (27.45%). The age distribution of ESBL producers in our study was similar across the 30-40, 50-60, and 70-80 year age groups, consistent with Khanfar et al. (2009), who found that 56% of ESBL isolates were from patients over 60 years. The increasing prevalence of ESBL- producing bacteria is likely due to factors such as self-medication, the use of suboptimal antimicrobial drugs, and poor hygiene practices (Walson et al., 2001).

**Conclusion**

This study at BPKMCH analyzed 142 samples, revealing bacterial growth in 35 samples. *E. coli* was the most prevalent pathogen (63%), followed by *Klebsiella* (26%). The highest growth (29.62%) was in the 60-70 age group, showing a significant association ($P<0.05$) with age. Polymyxin B was 100% effective against gram- negative

bacteria. Of the isolates, 22 (81.48%) were MDR, and 15% of 20 suspected ESBL producers tested positive, resulting in an overall ESBL prevalence of 11.11%.

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## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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