Multidrug resistant and extended spectrum β-lactamase producing gram negative bacterial uropathogens among females in a tertiary hospital, Pokhara

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ABSTRACT

Introduction: Extended spectrum β-lactamase producing gram negative bacilli has emerged and spread worldwide as an important cause of urinary tract infections and indicates a major threat. The study aimed to determine the prevalence of multidrug resistant and extended spectrum beta-lactamase producing gram negative bacterial uropathogens among females in a tertiary level hospital. **Methods:** A hospital-based cross-sectional study was conducted in Pokhara Academy of Health Sciences, Pokhara from February to November, 2019. A total number of 301 midstream urine samples were collected and examined using MacConkey agar and blood agar medium. Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method on Mueller Hinton agar using Clinical and Laboratory Standards Institute guidelines. **Results:** Out of 301 mid-stream urine samples, 99(33%) sample showed significant bacterial growth. Among them, 78(79%) were gram negative bacteria. Escherichia coli were the predominant organism. Multidrug resistant gram negative isolates were 65.4%. Among 78 Gram negative isolates, 31(39.7%) were extended spectrum β-lactamase producers. Among extended spectrum β-lactamase producers, 27(87.1%) were MDR. Highest frequency of extended spectrum β-lactamases production was seen in E. coli, 23(74.2%). Majority of gram negative bacteria showed susceptibility toward colistin and nitrofurantoin. Ampicillin was found to be highly resistant towards gram negative uropathogen. **Conclusions:** This study found that higher proportion of multi-drug resistants were among gram negative isolates and further more among extended spectrum β-lactamase producing gram negative isolates. Thus, there is urgent need to address the issue of antimicrobial resistant and promote rational use of the antibiotics in our region.

Keywords: Extended spectrum β-lactamase, gram negative isolates, multidrug resistant, urinary tract infection.

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INTRODUCTION

Urinary tract infection (UTI) is a type of microbial infection that affects the urinary tract. It is categorized as upper UTI or lower UTI on the basis of anatomical classification and symptomatic or asymptomatic UTI on the basis of clinical diagnosis.[1] Bacterial colony count $\geq 10^5$ CFU/ml is considered as significant bacteria.[2] Major causative agents of UTI are Escherichia coli, Klebsiella spp., Proteus spp., Staphylococcus aureus, coagulase negative Staphylococcus, Pseudomonas aeruginosa and Citrobacter spp.[3,4] Urinary tract infection (UTI) is the second most common bacterial infection, accounting for 25% of all the infections. It results from the invasion of the bacteria into the urinary tract, either from an endogenous source or an exogenous source. Females are more likely than males to develop UTI due to a shorter urethra, facilitating the bacteria to enter into the bladder more easily, closer proximity to the anus and the absence of prosthetic secretions.[3,5]

Every year, about 150 million people worldwide are diagnosed with UTI. Escherichia coli (E. coli) alone accounts for 80 to 90% of urinary tract infections.[6] In addition, antibiotic resistance of urinary tract pathogens has been known to increase worldwide, especially to commonly used anti-microbials. The pattern of antibiotic resistance may vary over time and also depends on the site of isolation and environmental conditions.[7] The majority of the problems associated with antimicrobial resistance have been shown to be due to the presence of transferable plasmids encoding multidrug resistance and their dissemination among different bacterial species.[8]

Multidrug resistant isolates have emerged as major complications in the therapeutic management of patients with infectious diseases.[9] The evolution of beta-lactamases mediated resistance by extended spectrum β-lactamase (ESBL)-producing bacteria has been associated with increased and irrational use of antibiotics, particularly the 3rd generation of cephalosporins.[1] ESBLs are defined as enzymes produced by certain bacterial pathogens that are capable of hydrolyzing penicillin, broad and extended spectrum cephalosporin and monobactams and are inhibited by clavulanic acid. In addition, ESBLs producing organisms are frequently resistant to many other class of antibiotics including aminoglycosides and fluoroquinolones. Thus, treatment of this infection is often a therapeutic challenge. ESBLs was first detected among Klebsiella spp and then later among E. coli, P. aeruginosa and Serratia spp. and other gram negative bacilli.[10]

ESBLs are plasmid-borne and evolved from point mutations that altered the configuration of the beta lactamases, active site. They were first isolated in 1983 in Germany.[11] Most ESBLs can be classified into 4 major groups: temoneira (TEM), sulfhdryl variant (SHV), cefotaximase munich (CTX-M) and oxacillinase (OXA). However, other groups of ESBLs, such as VEB, PER, GES, TLA, IBC, SFO-1, BES-1, and BEL-1, have also been reported.[12]

This study was undertaken to determine the prevalence of MDR and extended spectrum beta-lactamase producing gram negative bacterial uropathogens among females.

METHODS

This hospital-based cross-sectional study was carried out at the Pokhara Academy of Health Sciences, Pokhara, Nepal, from February to November 2019. A total of 301 midstream urine samples were collected in well-labelled, screw-capped sterile containers and examined using standard microbiological techniques. Samples from both symptomatic and asymptomatic females were included in the study. Urine collected from the catheter was excluded. The further processing of the sample was done according to standard procedures. Urine sample was cultured in MacConkey agar and blood agar medium by a semi-quantitative culture technique using a standard loop. Culture media were inoculated and incubated aerobically at 37°C for 24 hours. Following the incubation, the total number of colony-forming unit per millilitre (CFU/ml) of urine was estimated in accordance with the volume of urine inoculated previously, and the total count per millilitre was calculated.

The bacterial count was reported as following[1]: less than 10^4 CFU/ml organisms=insignificant bacteriuria, 10^4-10^5 CFU/ml organisms=low count significant bacteriuria, and more than 10^5 CFU/ml organisms=significant bacteriuria. On the culture plate showing significant growth, bacterial colony morphology, staining reaction, and biochemical properties were followed for identification of bacteria. Mueller Hinton Agar (MHA) was used for antimicrobial susceptibility testing by the Kirby Bauer disk diffusion method. Antibiotics were chosen according to Clinical and Laboratory Standards Institute (CLSI) guidelines. ampicillin (10 mcg), amikacin (30 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), ceftazidime-clavulanic acid (30 mcg/10 mcg), ciprofloxacin (5 mcg), colistin (10 mcg), imipenem (10 mcg) and nitrofurantoin (300 mcg) antibiotics were used. The plates were incubated at 37°C for 24 hours and examined. Sensitive, intermediate and resistance of the test organism to each antibiotics were noted.[13]

The multidrug resistance pattern of the isolates was identified by observing the resistance pattern of the isolates to the antibiotics of three or more than three classes.[4]

All MDR gram negative bacteria were tested for ESBLs. Isolates showing inhibition zone size ≤22 mm with ceftazidime and ≤27 mm with cefotaxime were identified as potential ESBL producers.[14] In this study, disc of ceftazidime (30mcg), disc of ceftazidime and clavulanic acid (30mcg/10mcg) were used for the confirmation of ESBLs producing strains. Discs were placed at 25mm apart, centre to centre, on a lawn culture of the test isolate on MHA plate and incubated overnight at 37°C. An increase of more than 5 mm in the diameter of the inhibition zone in combination with clavulanic acid versus its zone when tested with antibiotics alone confirmed ESBLs.[4,14]

Ethical approval was taken from the Institutional Review Committee (IRC), Pokhara University (Ref. no. 46/076/077). Informed consent was taken from the participants who

were enrolled in this study. Data entry was done in microsoft office excel 2013. Data cleaning and statistical analysis were done using statistical packages for the sciences (SPSS) version 20.0. Frequency and percentages were computed, and a diagrammatical presentation was done.

RESULTS

Out of 301 total samples included, 190(63.0%) samples showed no growth, 12(4.0%) showed insignificant bacterial growth and 99(33.0%) showed significant bacterial growth. As shown in Table 1, the majority of the participants in this study were in the age group of 21 to 30 years 109(36.2%), whereas age group less than 20 years was the least 30(10.0%). The higher proportion of bacterial growth was found in the age group of 21 to 30 which was 25(25.3%).

Table 1: Distribution of bacterial growth among different age group (n=301)

Age group (in years)	Bacterial Growth		Total
	No, n (%)	Yes, n (%)	
≤ 20	22(10.9)	8 (8.0)	30 (10.0)
21-30	84 (41.6)	25(25.3)	109 (36.2)
31-40	48 (23.7)	21 (21.2)	69 (22.9)
41-50	25 (12.4)	20 (20.2)	45 (15.0)
≥ 51	23(11.4)	25 (25.3)	48 (15.9)
Total	202 (100)	99 (100)	301 (100)

Table 2 shows that there were 21(21%) gram positive and gram negative 78(79%) bacterial species isolated in urine. Out of 78 gram negative isolates, 51 (65.4%) isolates showed multidrug resistance. In this study, among 31 ESBLs producers 27(87.1%) were MDR and 4(12.9%) were Non-MDR and among 47 ESBLs Non- producers 24(51.1%) were MDR and 23(48.9%) were Non-MDR.

Table 2: Proportion of gram negative isolates and ESBL producer and non-producer with MDR and Non-MDR

Type of isolates (n=99)	Non-MDR, n (%)	MDR, n (%)	Total n (%)
Gram positive	10 (47.6)	11(52.4)	21 (100.0)
Gram negative isolates (n=78)	27 (34.6)	51(65.4)	78 (100.0)
Gram negative ESBLs (n=78)			
ESBLs producer	4(12.9)	27(87.1)	31 (100.0)
ESBLs Non-producer	23(48.9)	24(51.1)	47 (100.0)

Gram negative isolates were highly sensitivity to colistin (99%), imipenem (77%), nitrofurantion (77%), amikacin (72%) and ceftazidime (45%) with ampicillin (24%) having the least sensitivity. Three isolates were sensitive to all antibiotics tested as shown in Figure 1.

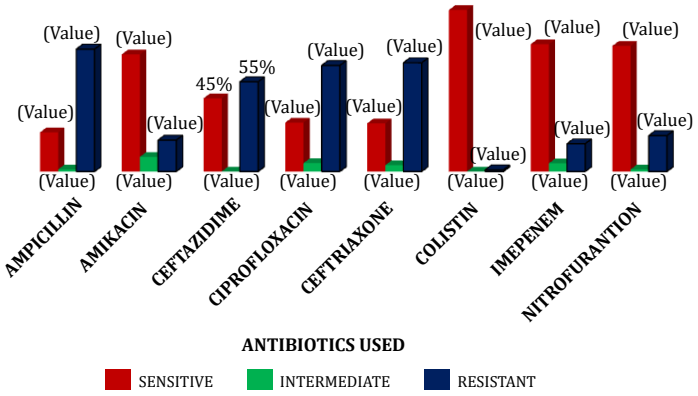

Figure 1: Antibiotic sensitivity test of gram negative isolates

Table 3 shows the distribution of identified bacteria by gram reaction and ESBL producer. All the gram negative isolates were screened for ESBLs. Among the 78 gram negative isolates, 31(39.7%) were ESBL producer and 47(60.3%) were non-ESBL producer. Among ESBL producers, highest frequency of ESBL production was seen in E. coli 23 (74.2%) isolates.

Table 3: Distribution of gram negative bacteria on the basis of ESBL producer and non-producer

Type of gram negative bacteria (n=78)	ESBL producer n (%)	ESBL non-producer n (%)	Total
E. coli	23 (74.2)	20 (42.6)	43 (55.1)
K. pnuemoniae	3 (9.7)	9 (19.1)	12 (15.4)
Acinetobacter spp	2(6.5)	6 (12.8)	8 (10.3)
K. oxytoca	1(3.2)	3 (6.4)	4 (5.1)
P. aeruginosa	1(3.2)	3 (6.4)	4 (5.1)
P. vulgaris	1(3.2)	1 (2.1)	2 (2.6)
Enterobacter spp	0	3 (6.4)	3 (3.8)
C.furundii	0	2 (4.2)	2 (2.6)
Total	31 (39.7)	47 (60.3)	78 (100)

Among 31 ESBLs producing gram negative isolates, the highest sensitivity was found (74%). Only about three-fifth isolates were sensitive with imipenem and the least sensitivity was found with ampicillin (3.0%) (Figure 2).

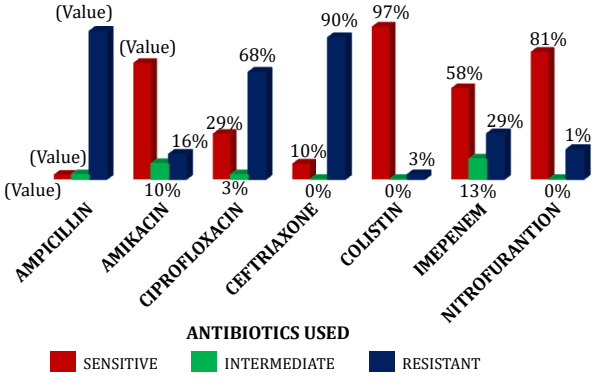

Figure 2: Antibiotics sensitivity test of ESBLs producer gram negative isolates

Among 23 ESBL producer E. coli isolates, the highest sensitivity was found with colistin (96.0%), followed by nitrofurantoin (83.0%) and amikacin (70.0%). Only half of the isolates were sensitive with imipenem (52.0%) and 4% with ceftriaxone. None of isolates were sensitive with ampicillin as shown in Figure 3.

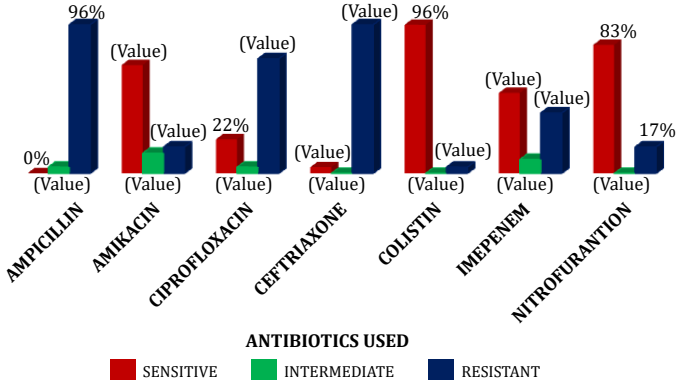

Figure 3: Antibiotics sensitivity test of ESBLs producing E. coli

Among four ESBLs producing Klebsiella spp, the highest sensitivity was found with nitrofurantoin (100%), colistin (100%), followed by imipenem (75%), amikacin (75%). Only one -fourth of the isolates were sensitive with ceftriaxone and ciprofloxacin. None of the isolates were sensitive with ampicillin (Figure 4).

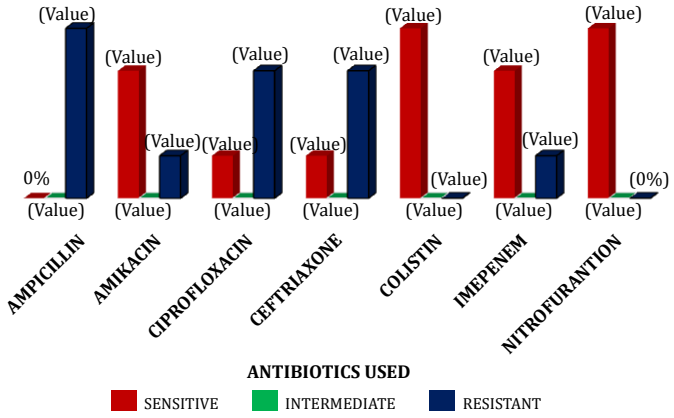

Figure 4: Antibiotics sensitivity test of ESBLs producing Klebsiella spp.

DISCUSSION

The study examined patterns of multidrug resistant and extended spectrum beta-lactamases producing gram negative bacterial uropathogens among females in a tertiary level hospital. Among total 99 isolates, about 79% were gram negatives isolates. Gram negative isolates were examined for multidrug resistance and ESBL production in the study. Among gram negatives isolates, the most common isolate was E. coli i.e. 43 (55.5%) followed by Klebsiella spp. This means E. coli is one of the most common bacteria causing UTI in females. A study by Thapa et al.[15] also found that E. coli was the predominant bacterial pathogen i.e. 65.1%. In contrast to the study, higher proportion of E coli was found in a study by Tiwari et al.[4] i.e.70%. Based on the ESBL production, 39.7% Gram negative isolates were ESBLs producers; and among ESBL producer isolates also, the most common isolates was E. coli i.e. 23 (74.2%) which indicates the risk of treatment failure by beta-lactam antimicrobials.

The age group analysis of microbial growth shows that the females of 20-30 years had highest proportion of UTI (25.3%). This proportion is similar to the study done by Thapa et al.[15] which found that 27.8% UTI was in the age group of 21 to 30. The study found that E. coli was predominant gram negative isolate followed by Klebsiella pneumoniae. Among total gram negative isolates, prevalence of E .coli was 55.1% in the study. Similar to this study, previous studies by Thapa et al. (55.3%) and Kattel et al. (59.6%) also reported that E. coli was the most frequently isolated uropathogen.[16,17] However, slightly higher prevalence was reported in a study by Bhandari et al that showed 63.4% gram negative isolates was E. coli followed by Klebsiella pneumoniae with 8% isolates.[5] The study also found that 74.2% ESBL producing isolates were E.coli. It means that among ESBL producing gram negative isolates, about three-fourth (74.2%) were E. coli only. Similar to this study, a study conducted by by Poudyal et al.[18] also found that E.coli (80.0%) was the major ESBL producer followed by Klebsiella pneumonia.

Among the common antibiotics used against all the gram negative isolates, colistin showed higher susceptibility (99.0%) followed by imipenem (78.0%), and nitrofurantoin (77.0%). Similarly, a study by Thapa et al.[16] revealed that nitrofurantoin was one of the most effective antibiotics for gram negative bacteria (78%). In contrast to this study, higher proportion of gram negative isolates i.e. 93.5% were sensitive to nitrofurantoin in the previous study conducted in 2017 in a tertiary care hospital of Nepal.[5] On the other hand, about–three-fourth of the gram negative isolates were resistant to ampicillin in the present study. The study shows that imipenem, ceftriaxone and ampicillin were less effective for gram negative isolates. About half of E. coli isolates were sensitive to imipenem, only 4% with ceftriaxone and none of isolates were sensitive with ampicillin. A previous study also found that 87.1% gram negative isolates were resistant to ampicillin.[5] Although this proportion is higher as compared to our study, important meaning is that ampicillin has limited sensitivity to gram negative isolates.

In our study, 65.4% gram negative isolates were MDR strains. Almost, similar results of MDR have been reported by Poudyal et al.[18] i.e. 64.6%, Bhandari et al.[5] i.e.73.2% and Thapa et al.[16] i.e.73%. However, higher prevalence of MDR was reported by Ullahet al.[6] which was 83.0%, and by Ansari et al.[19] i.e. 78.0%. At the same time, a study by Awasthi et al.[20] has found the lower prevalence of MDR i.e.42.8%. Higher MDR results might be due to many factors including misuse of antibiotics by the health care professionals, non-skilled practitioners as well as self-medication practice of general public and also inadequate surveillance system.

Among 78 gram negative isolates, 65.5% were MDR; and among ESBL producer gram negative isolates, the proportion of MDR was further higher that i.e. 87.1% which is the most important finding of the study. Among 78 gram negative isolates, 31(39.7%) of isolates were ESBLs producer. Almost similar results were obtained by Giwa et al. that shows the 34.3% of ESBLs producer.[21] The higher prevalence of ESBL was shown by Akram et al.[22] i.e. 42.0% and Sankar et al.[23] i.e. 48.5%. In contrast to the above findings, lower prevalence of ESBL was reported by Kader et al. which was only 4.8%.[24] The prevalence of ESBLs among clinical isolates varies from country to country and from institution to institution and these differences may be due to geographical variations, local antibiotic prescribing habits.[24]

Among 31 ESBLs producers, E. coli attributes highest prevalence (74.2%) followed by K. pneumonia (9.7%) in the study. Our finding is similar with the finding of the study of Tiwari et al.[4] i.e. 75.8%. In contrast to the study, higher prevalence of ESBLs producing E. coli were reported by Poudyal et al.[18] i.e. 80%, Hassan et al.[25] i.e. 85%. However, lower prevalence of ESBLs E. coli was reported by Thapa et al.[16] i.e. 7.6%. The study conducted by Khadri et al.[26] reported the higher prevalence of ESBLs producing Kleibsiella spp i.e. (34.1%) than E. coli i.e. (30.3%). We can conclude that E. coli was the highest among ESBL producing gram negatives isolates.

According to our study, 27(87.1%) ESBLs producing isolates were found to be MDR whereas only 24(51.1%) ESBLs non-producing isolates were MDR. Higher MDR patterns among ESBL producer might be due to higher plasmid mediated resistance to antimicrobial agents, alteration of target site of antibiotics, impermeability of antibiotics etc.[27] ESBLs producing E.coli shows higher sensitivity to Colistin i.e. (96.0%) followed by nitrofurantoin i.e. (83.0%) and amikacin (70.0%). This result is supported by Thapa et al.[16] and highly resistant to ampicillin and ceftriaxone which is similar to the study done by Chander et al.[28]

A higher proportion of ESBL-producing gram negative isolates and limited sensitivity to some antibiotics such as ceftriaxone and ampicillin indicate that drug resistance is increasing, and drug prescription should be based on the sensitivity test result.

The limitation of this study was that only phenotypic characteristics of organisms were studied; and species - subspecies were not isolated.

CONCLUSIONS

Among gram negative isolates, about two-fifth isolates were ESBLs producers and E. coli was the most common ESBLs producing bacteria. More importantly, about 90% of ESBLs producers were MDR. Gram negative isolates showed higher susceptibility with colistin and nitrofurantoin; and imipenem, ceftriaxone and ampicillin were less effective for gram negative isolates. Only half of E. coli isolates were sensitive to imipenem, only 4% with ceftriaxone and none of isolates were sensitive with ampicillin, which indicates the major future threat of drug resistant in Nepal. Findings of the study show that there is urgent need to address the issue of antimicrobial resistance. Antibiotics should be prescribed only after performing the antimicrobial susceptibility test to decrease the resistance. ESBLs testing should be adopted as a routine laboratory test.

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