

Microbiology

OCCURRENCE OF EXTENDED SPECTRUM β - LACTAMASE PRODUCING *Escherichia coli* AND *Klebsiella* spp AMONG MULTIDRUG RESISTANT ISOLATES FROM URINARY TRACT INFECTION PATIENTS

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Abstract

Antimicrobial resistance along with multi drug resistance (MDR) among common bacterial pathogens have resulted into treatment failures and increased economic burden of health care system world around. Control of these pathogens necessitates their early and reliable detection. The aim of this study was to study the prevalence of extended spectrum β -lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* spp. among the MDR isolates from UTI patient and to determine the antimicrobial susceptibility pattern of them. During the study period, a total of 520 urine samples from the clinically defined urinary tract infection (UTI) patients visiting B.P. Smriti Hospital were collected and processed. Isolates were identified by conventional microbiological procedures. Antimicrobial susceptibility test (AST) was performed by Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA) and results were interpreted according to CLSI 2013. MDR isolates were screened for possible ESBL production using Ceftazidime and Cefotaxime disk. Suspected ESBL producers were subjected to combined disk (CD) assay for confirmation of ESBL production using MASTDISK™ ID ESBL detection discs consisting of Ceftazidime and Ceftazidime plus Clavunic acid. A total of 138 isolates were obtained among which *Escherichia coli* (95 i.e. 68.8%) predominated *Klebsiella* spp. The prevalence of MDR among urinary *E. coli* and *Klebsiella* spp. isolates were found to be high i.e. 64.2 % (61/95) and 53.5% (23/43) respectively. The first line drugs, Nitrofurantoin followed by Amikacin was found to be most effective for both *E. coli* and *Klebsiella* spp. High prevalence of total ESBL producers (43/84; 51.91%) among the tested MDR isolates were statistically significant. Thus higher rate and statistically significance of ESBL production prevalence over MDR isolates underline the necessity for routine ESBL detection.

Keywords: ESBL, AST, *E. coli*, *Klebsiella* spp, MDR

Introduction

Urinary tract infection are considered to be the most common bacterial infections (Foxman 2002) and it has been estimated that up to 150 million people are affected annually by this disease (Aboderin *et al.* 2009). *Escherichia coli* is the most notorious causative agent of UTI followed by *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* (Echols *et al.* 1999; Czaja *et al.* 2007).

Irrational use of antibiotics, increased uses of antibiotics in animal and plants, unhygienic

practices, incompetency to adopt proper infection control technique alongside with accessibility of antibiotics without prescription and counterfeit products of doubtful quality in developing countries have contributed antimicrobial resistance (ASM 2009). Multi drug resistance (MDR) is defined as resistance to two or more than two of the antimicrobial agents belonging to different classes (CDC 2006).

β -lactamases are the major defense of Gram negative bacteria against β -lactam antibiotics (Jacoby and Munoz-Price 2005). ESBL are plasmid-mediated bacterial enzymes that confer

resistance to the penicillins (except temocillin), first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) and are inhibited by β -lactamase inhibitors such as clavulanic acid (Paterson and Bonomo 2005). Different studies conducted at different hospital around Kathmandu, capital of Nepal, over the span of last decade (2004 through 2014) reveals that ESBL producers are increasing (Pokharel 2004; Manandhar *et al.* 2006; Shrestha 2009; Poudyal 2010; Adhikari 2011; Dhakal *et al.* 2012; Chander and Shrestha 2013).

Materials and Methods

The study was carried out at B.P Smriti Hospital from April to September 2013. During this period, a total of 520 urine samples from patients suspected of UTI were collected and processed according to the standard laboratory methods described by Cheesbrough (2006).

Sample collection and transportation

The patients were given a clean, dry and sterile and leak proof container and requested for 5-10 ml mid-stream urine (MSU) sample. Before providing the container, each patient was instructed properly for the collection of sample. The samples with proper labeling were processed as soon as possible. But if urine could not be tested and cultured within 4 hours of collection, the sample were refrigerated at 4°C (NICE 2007). Urine can be stored in the refrigerator for up to 24 hours (Tanagho and Meaninch 2004).

Culture of specimen

Culture of each urine sample was done on Blood Agar (BA) plate and MacConkey Agar (MA) plate. An inoculating loop calibrated to deliver 0.001ml of uncentrifuged urine was used for inoculation. After inoculation the plates were incubated aerobically at 37°C for 24 hours.

Identification of isolates

Identification of the isolates were done by using microbiological techniques as described in the Bergey's manual which involves morphological appearance of the colonies, staining reactions and

various biochemical properties. Isolated colonies from pure culture were identified by appropriate biochemical tests and identified accordingly (Cheesbrough 2006; Forbes *et al.* 2007).

Antimicrobial susceptibility testing

Antimicrobial Susceptibility tests of different clinical isolates were performed by Kirby-Bauer disk diffusion method using Mueller Hinton Agar (MHA) following Clinical and Laboratory Standard Institute guidelines (CLSI 2013).

Screening and confirmation of ESBLs

MDR isolates were screened for possible ESBL production using Ceftazidime (30 μ g) and Cefotaxime (30 μ g) (CLSI 2013). According to the guidelines, the MDR showing reduced susceptibility to one or all of these drugs with zone of inhibition diameter for ceftazidime ≤ 22 mm and cefotaxime ≤ 27 mm were considered as the possible ESBL producing strains. The suspected ESBL isolates were tested for confirmation by using the Combination Disc Method, using Ceftazidime (30 μ g) and ceftazidime (30 μ g) plus clavulanate (10 μ g). An increase in zone diameter of ≥ 5 mm in the presence of clavulanate was confirmed as ESBL producers.

Data Analysis

All the results were entered in the worksheet of statistical package for social science (SPSS) software (Version 16.0). Chi-squared test was used to determine significant association of MDR with sex, significant growth with sex and ESBL with MDR. Mean age along with standard deviation and sex ratio were also calculated.

Results

Out of total 520 urine samples collected from patients suspected of UTI, 206 (39.61%) were from male patients and 314 (60.39%) from female patients. In the case of male patients, 54 (26.21%) of 206 urine sample showed significant growth and in case of female patients, 84 (73.79%) of 314 showed significant growth (figure 1).

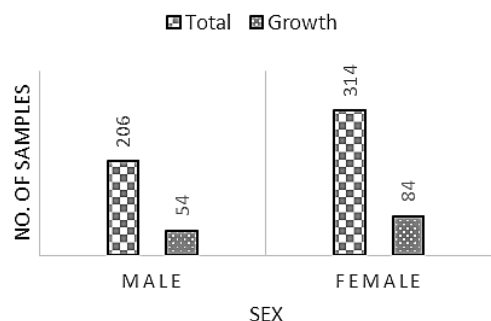


Figure 1. Sex-wise distribution of sample and growth.

While the ratio of female to male for the distribution of sample was 1.52:1, the ratio of female to male for the distribution of growth was 1.55:1. The association of significant growth in male and female patients was determined by chi-squared test and it was found that higher proportion of significant growth among female patient is statistically insignificant ($p>0.01$).

Distribution of Urinary isolates

Of the 138 samples showing significant growth, 95/138 (68.8%) were *E. coli* and 43/138 (31.2%) were *Klebsiella* spp. Among the 84 significant growth cases from female patients, the highest number of isolates (17.4%) was obtained from age group 21-30 years while in male patients highest number of isolates (9.4%) was found in age group 61-70 years out of 54 significant growth cases.

The least number of isolates (1.4%) was obtained from age group above 80 years in female patients whereas none of the isolates were obtained from male patients of age group 0-10. The age of the patients having growth ranged from 1 to 88 years with the mean age of 44.05 ± 20.12 (Mean $\pm$ SD).

Antibiotic susceptibility pattern of *E. coli* and *Klebsiella* spp.

Nitrofurantoin and Amikacin were found to be more effective against *E. coli* with susceptibility of 92.6% and 85.3% respectively, whereas; Amoxycillin, Nalidixic acid, Ofloxacin and Ceftriaxone were found to be the least effective

drugs with susceptibility of 1.1%, 9.5%, 16.8% and 28.4% respectively.

Table 1. Age and sex-wise distribution of significant growth.

Age	Male	Female	Total
0-10	0 (0.0%)	3 (2.2%)	3 (2.2%)
11-20	1 (0.7%)	8 (5.8%)	9 (6.5%)
21-30	8 (5.8%)	24 (17.4%)	32 (23.2%)
31-40	12 (8.7%)	15 (10.9%)	27 (19.6%)
41-50	4 (2.9%)	11 (8.0%)	15 (10.9%)
51-60	7 (5.1%)	7 (5.1%)	14 (10.1%)
61-70	13 (9.4%)	11 (8.0%)	24 (17.4%)
71-80	5 (3.6%)	3 (2.2%)	8 (5.8%)
80>	4 (2.9%)	2 (1.4%)	6 (4.3%)
Total	54 (39.1%)	84 (60.9%)	138 (100.0%)

MDR Pattern

Of the total 138 isolates, 84 (60.9%) were MDR while other 54 (39.1%) were non-MDR strains. Of the 84 MDR cases, 61 were *E. coli* and the remaining 23 were *Klebsiella* spp. Higher distribution of MDR isolates were found in female patients (54.8 %, 46/84) compared to that of male patients (45.2 %, 38/54).

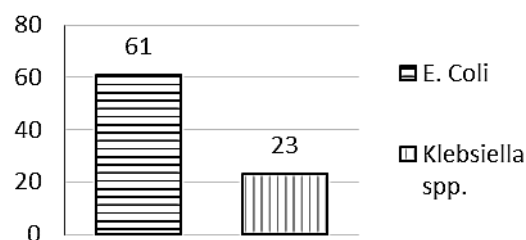


Figure 2. Distribution of MDR strains among Causative Agents.

The association of MDR and non-MDR strains with sex was determined by chi-square test and found that higher proportion of MDR in female patient was statistically insignificant ($p>0.01$).

Table 2. Antibiotic susceptibility pattern of *Escherichia coli*.

Antibiotic Used	Sensitive	Intermediate	Resistant
Amoxycillin	1 (1.1%)	5 (5.2%)	89 (93.7%)
Nalidixic Acid	9 (9.5%)	5 (5.2%)	81 (85.3%)
Ofloxacin	16 (16.8%)	68 (71.6%)	11 (11.6%)
Norfloxacin	42 (44.2%)	28 (29.5%)	25 (26.3%)
Nitrofurantoin	88 (92.6%)	5 (5.2%)	2 (2.2%)
Amikacin	81 (85.3%)	13 (13.6%)	1 (1.1%)
Ciprofloxacin	27 (28.4%)	42 (44.2%)	26 (27.4%)
Cefixime	41 (43.1%)	21 (22.1%)	33 (34.8%)
Ceftriaxone	27 (28.4%)	19 (20.0%)	49 (51.6%)
Cotrimoxazole	62 (65.2%)	8 (8.4%)	25 (26.4%)
Cefotaxime	48 (50.6%)	5 (5.2%)	42 (44.2%)
Gentamycin	58 (61.0%)	36 (37.9%)	1 (1.1%)
Ceftazidime	48 (50.6%)	6 (6.2%)	41 (43.2%)

In case of *Klebsiella* spp, Nitrofurantoin, Amikacin, Gentamycin, and Cotrimoxazole were found to be more effective with 95.4%, 83.8%, 69.8% and 60.4% susceptibility respectively, whereas; Amoxycillin, Nalidixic acid, Ofloxacin, Ceftriaxone and Ciprofloxacin were found to be the least effective drugs with susceptibility of 2.3%, 6.9%, 30.2%, 30.2% and 30.2% respectively.

ESBL production among MDR isolates

Out of 61 MDR *E. coli* and 23 MDR *Klebsiella* spp., 47 isolates (77.0%) and 12 isolates (52.2%) were found to be positive on primary ESBL screening test respectively.

Among the screened positive isolates, 8/12 (66.7%) *Klebsiella* spp. and 35/47(74.5%) *Escherichia coli* were confirmed as ESBL producers. Out of total 84 MDR strains, 43 (72.9%) were confirmed as ESBL isolates.

Table 3: Antibiotic susceptibility pattern of *Klebsiella* spp.

Antibiotic Used	Sensitive	Intermediate	Resistant
Amoxycillin	1 (2.3%)	2 (4.6%)	40 (93.1%)
Nalidixic Acid	3 (6.9%)	4 (9.3%)	36 (83.8%)
Ofloxacin	13 (30.2%)	27 (62.9%)	3 (6.9%)
Norfloxacin	18 (41.8%)	10 (23.2%)	15 (35.0%)
Nitrofurantoin	41 (95.4%)	1 (2.3%)	1 (2.3%)
Amikacin	36 (83.8%)	6 (13.9%)	1 (2.3%)
Ciprofloxacin	13 (30.2%)	22 (51.2%)	8 (18.6%)
Cefixime	17 (39.5%)	7 (16.2%)	19 (44.3%)
Ceftriaxone	13 (30.2%)	16 (37.2%)	14 (32.6%)
Cotrimoxazole	26 (60.4%)	2 (4.6%)	15 (35.0%)
Cefotaxime	31 (72.2%)	3 (6.9%)	9 (20.9%)
Gentamycin	30 (69.8%)	13 (30.2%)	0 (0.0%)
Ceftazidime	31 (74.5%)	5 (9.3%)	7 (16.2%)

Among the total 43 ESBL producing strains, the overall highest number of ESBL producing strains was found in the age group of 21-30 years, i.e., 25.6% of total ESBL, (11/43).

ESBL producing strains were found comparatively higher in female patients of age group 21-30 (18.6% i.e., 8/43) than that of male patients with same age group (7.0%, i.e. 3). The ESBL producing strains were not observed from male patients of age group 11-20 years and from both male and female patients of the age group 0-10 years.

Discussion

Out of total 520 samples (included only from outpatients), only 138 (26.5%) samples showed significant growth, whereas the remaining samples, i.e. 382 (73.5%) showed no growth. Varying significant growth has been found across different institute around the globe.

Table 4: Age group and sex wise distribution of ESBL strains

Antibiotic Used	Sensitive	Intermediate	Resistant
Amoxycillin	1 (2.3%)	2 (4.6%)	40 (93.1%)
Nalidixic Acid	3 (6.9%)	4 (9.3%)	36 (83.8%)
Ofloxacin	13 (30.2%)	27 (62.9%)	3 (6.9%)
Norfloxacin	18 (41.8%)	10 (23.2%)	15 (35.0%)
Nitrofurantoin	41 (95.4%)	1 (2.3%)	1 (2.3%)
Amikacin	36 (83.8%)	6 (13.9%)	1 (2.3%)
Ciprofloxacin	13 (30.2%)	22 (51.2%)	8 (18.6%)
Cefixime	17 (39.5%)	7 (16.2%)	19 (44.3%)
Ceftriaxone	13 (30.2%)	16 (37.2%)	14 (32.6%)
Cotrimoxazole	26 (60.4%)	2 (4.6%)	15 (35.0%)
Cefotaxime	31 (72.2%)	3 (6.9%)	9 (20.9%)
Gentamycin	30 (69.8%)	13 (30.2%)	0 (0.0%)
Ceftazidime	31 (74.5%)	5 (9.3%)	7 (16.2%)

Previous studies in Nepal have reported as low as 9.34 % significant growth (Chander and Shrestha 2013) and as high as 26.4% (Manandhar *et al.* 2006). Significant growth of 19.61%, 23.9% and 23.41% were reported by Poudyal (2010), Adhikari (2011) and Dhakal *et al.* (2012) respectively. Manandhar (1996) has stated the possible cause of low rate growth positivity may have been attributed by the fact that samples might be from patients under treatment, infection due to slow growing organisms or due to those organisms that were not able to grow on the routine culture media used.

Many pathogenic bacteria are isolated in the urinary tract infections. But since our study is focused on the prevalence of ESBL producing *Escherichia coli* and *Klebsiella* spp. only, other isolated bacteria were not taken into consideration in this study. *Escherichia coli* and *Klebsiella* spp. only were considered owing to their ability to produce ESBLs in large quantity (Babypadmini and Appalaraju 2004).

In agreement with the earlier studies, this study also showed relatively higher number of female patients (60.4%) requesting urine culture than male patients (39.6%). Following the similar trend for urine culture, growth rate also was higher among females (84/138, 60.9%) than males (54/138, 39.1%). Similar results were reported by Bomjan (2005) and Gupta (2010). The higher urine culture positivity among female patients has been attributed to the physiological status of the body; the proximity of anus and the urethral opening in females is really short and female patients depending on their hygienic habits are highly prone to urinary tract infection (Harvey 2013).

In addition, inclusion of higher number of female patient (60.38%) of female patients too may have contributed for the higher growth rate among them. Resembling the outcomes of the result by Gupta (2010), this study also showed higher prevalence of *E. coli* (68.8 %) than *Klebsiella* spp.

Amongst the various drugs used against *Escherichia coli* isolated from the urine samples, Nitrofurantoin and Amikacin were found to be more effective drugs but Amoxycillin, Nalidixic acid and Ofloxacin were found to be the least effective drugs. Nitrofurantoin with few other antibiotics represents effective options for the empiric therapy of patients with uncomplicated lower UTI (Wagenlehner *et al.* 2009).

Thakur *et al.* (2013) showed Amoxycillin to be the least susceptible drug followed by Ciprofloxacin with susceptibility of 12.2% and 35% respectively. Since Amoxycillin is the first line drug which is easily hydrolyzed by the bacterial enzymes and offer less in the treatment of Gram negative bacterial infections, it was found to be the least effective drug.

Out of 138 *E. coli* and *Klebsiella* spp. isolates, 84 MDR strains were screened for the ESBL production. Subsequently, 59 were suspected as possible ESBL producers. Following the Combination Disc Testing method 43 of the 59 suspected cases (72.9 %) were confirmed to be ESBL producers. Shrestha (2009) found that *E. coli* exhibited the highest rate of ESBL (88 out of 174 primarily screened isolates i.e. 50.57% which is similar to the result in our study.

In a similar study Buda (2010) reported out of 316 *E. coli* only 97 were suspected as ESBL producers on primary screening, however only 80 of them gave positive confirmatory test.

Every research work has its own limitation. No study can cover all the aspect of study area. This study tried to encompass more parameters on the prevalence of ESBL producing *E. coli* and *Klebsiella* spp. in MDR isolates from UTI patients. However this study may have been limited to collection of urine samples from the patient defined by physician as probable case of UTI.

The urine samples provided to us by the patient were assumed to be MSU sample and could be a limitation to our study. The study is limited to the collection of data at B.P Smriti Hospital from April to September of 2013. The urine samples were collected from the out-patient only which could be another limitation to our study.

Conclusion

Escherichia coli along with *Klebsiella* spp., the major pathogens causing UTI, were isolated in our study and its antibiotic susceptibility pattern and ESBL production were studied. The prevalence of MDR among urinary *E. coli* and *Klebsiella* spp. isolates were found to be high respectively.

Among the MDR isolates, high prevalence of total ESBL producers was observed. In this study, the first line drugs, Nitrofurantoin followed by Amikacin was found to be the most effective for both *E. coli* and *Klebsiella* spp.

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