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Research Article

EXTENDED SPECTRUM BETA LACTAMASES DETECTION AND MULTIPLE ANTIBIOTIC RESISTANCE INDEXING OF *ESCHERICHIA COLI* FROM URINE SAMPLES OF PATIENTS FROM A REFERRAL HOSPITAL OF EASTERN NEPAL

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Abstract

Background: *Escherichia coli* is the most common causative agent of urinary tract infection. Antibiotic resistance among uropathogens has become a prominent public health problem. Multidrug resistance bacteria have limited the therapeutic possibilities by producing Extended Spectrum Beta Lactamases (ESBL). **Objective:** Since routine monitoring of ESBL producers are not conducted in clinical laboratories their true prevalence is still unknown. So the objective of this research was to assess multiple antibiotic resistance (MAR) indices and determine ESBL production among *Escherichia coli* isolated from urine samples. **Methods:** Standard microbiological techniques and antibiotic sensitivity test were performed by Kirby Bauer disc diffusion method to identify *E. coli*. ESBL screening was done by using Ceftriaxone, Aztreonam, Cefotaxime, Ceftazidime and Cefpodoxime whereas confirmation by combined disc assay. SPSS 16 software was used to analyze data. **Results:** 86.95% *E. coli* isolates were MDR strains. 27 isolates had multiple antibiotic resistance (MAR) index of 0.2 and 5 isolates had MAR index of 0.7. *E. coli* isolates showed higher degree of resistance towards Amoxicillin (100%) while 100% were sensitive towards Gentamicin followed by Nitrofurantoin (62.31%). The reliable screening agent for ESBL detection with sensitivity 100% and positive predictive value of 80% was Cefotaxime. Combined disc assay detected 12/69 (17.31%) of *E. coli* isolates as confirmed ESBL producers. **Conclusion:** The ubiquity of ESBL-producing *E. coli* was observed emphasizing the necessity of regular surveillance of ESBL producing clinical isolates in clinical samples to minimize multi-drug resistance strains and avert the ineffectiveness of antimicrobial agent for good health practices.

Key words: Urine; *Escherichia coli*; ESBL; Multiple Antibiotic Resistance (MAR) index; MDR

Introduction

Urinary tract infection (UTI) is a common bacterial disease prevalent in community. *E. coli* accounts for 75.0-90.0% of all UTIs (Dromigney *et al.*, 2005). UTI is a common disease prevalent among Nepalese population (Kattel *et al.*, 2008).

Antimicrobial therapy of UTI caused by *E. coli* has been continually weakened due to the resistance against beta lactam antibiotics. β-lactamases are the major defense of Gram negative bacteria against β-lactam antibiotics (Jacoby *et al.*, 2005). Extended spectrum β-lactamases (ESBLs) are defined as the plasmid-mediated bacterial enzymes granting resistance to the penicillins (except temocillin), first, second and third-generation cephalosporins, and aztreonam (except cephamycins or carbapenems) but inhibited by β-lactamase inhibitors such as clavulanic acid (Paterson *et al.*, 2005). MAR index helps to assess the spread of bacterial resistance to more than three antibiotics (Krumperman, 1983).

The emergence of MDR and ESBL *E. coli* pose antibiotic management problems (Lim *et al.*, 2009). Multiple antibiotic resistances in bacteria and production of ESBL is most commonly associated with the presence of plasmids which contain one or more resistance genes, each coding a single antibiotic resistance phenotype (Paterson *et al.*, 2005; Daini *et al.*, 2008).

This study was designed to estimate the current prevalence and antimicrobial resistance patterns along with ESBL producing urinary isolates of *E. coli* among urinary tract infection patients visiting Bijayapur Hopsital, Dharan, Nepal.

Methodology

In a descriptive cross-sectional study conducted from March to August 2014, a total of 752 urine samples from suspected UTI patients visiting Bijayapur Hospital, Dharan were processed in the laboratory of Sunsari Technical College, Dharan for the isolation of *E. coli*. Informed consent was obtained for each sample used in the study from

the patients. Each sample was mixed well and aseptically inoculated on MacConkey agar plates and incubated at 37° C for 24 hours aerobically. Significant UTI was defined as the presence of $>10^5$ colony forming unit (CFU)/ml in the culture.

Further identification of *E. coli* was done by their cultural characteristics, Gram stain and different biochemical reactions. The antimicrobial susceptibility testing (AST) of *E. coli* isolates was done by Kirby Bauer Disc Diffusion Method as per CLSI guideline (CLSI, 2013). *E. coli* ATCC 25922 was used as a reference strain.

Multiple antibiotic resistance (MAR) index was determined using the formula MAR=a/b, where "a" denotes the number of antibiotics to which test isolate showed resistance and "b" is the total number of antibiotics employed for sensitivity (Akinjogunla *et al.,* 2010). An isolate was considered to be Multidrug Resistant (MDR) when it showed resistance to two or more drugs of different structural classes.

The test inoculum of 0.5 McFarland was carpet cultured on Mueller-Hinton agar. The screening agents, viz. Ceftriaxone (30µg), Cefpodoxime (10µg), Ceftazidime (30µg), Aztreonam (30µg), and Cefotaxime (30µg) were placed onto the inoculated media and incubated at 37^0C for 18-24 hours. Isolates showing Cefpodoxime <17mm, Cefotaxime <27 mm, Ceftazidime <22 mm, Aztreonam <27 mm, and Ceftriaxone <25 mm were suspected as possible ESBL producers (CLSI, 2013).

All the processed *E. coli* isolates were subjected to phenotypic confirmatory test using Combined Disks Assay consisting Ceftazidime (30µg) and Ceftazidime (30µg) plus Clavulanic acid (10µg) and Cefotaxime (30µg) and Cefotaxime (30µg) plus Clavulanic acid (10µg). An increase in zone diameter of ≥5mm in the presence of Clavulanate from either of the combination discs confirmed ESBL isolates (CLSI 2013). Data collected was analysed by using SPSS. P-value ≤0.05 was considered to be statistically significant.

Results

Out of 105 positive isolates, the overall prevalence of *E. coli* was found 69.6% in total 99 Gram negative isolates. Out of 69 *E. coli* isolates, 60 (86.95%) were multiple drug resistance and 12 (17.3%) isolates were found to be ESBL producers. The isolates were highly sensitive to Gentamicin and Tobramycin (100%) followed by Nitrofurantoin (62.31%). All the 69 isolates of *E. coli* were resistance towards Amoxicillin (Table 1).

Nine multidrug resistance patterns were observed in *E. coli* for the seven antimicrobial agents tested. Resistance to Amx-Cz was the most frequent pattern observed in 41.7% of *E. coli* isolates, 8.3% of *E. coli* isolates showed Amx-E-Cz-NA-Nit resistant pattern (Table 2). The MAR index ranges from 0.14 to 0.71. Out of 69 *E. coli* isolates, only 9 showed MAR index of 0.1 (< 0.2). 5 isolates showed MAR index of 0.7 i.e. these isolates were resistance to five antibiotics used in the testing (Table 3).

Table 1: Antibiotic susceptibility profile of *E. coli*

Antibiotic used	Sensitive		Resistant	
	Number	%	Number	%
Amoxicillin	0	0	69	100
Erythromycin	18	26.08	25	36.23
Tobramycin	69	100	0	0
Nitrofurantoin	43	62.31	18	26.08
Gentamicin	69	100	0	0
Cefazolin	7	10.14	56	81.15
Nalidixic acid	26	37.68	27	39.13

Table 2: Antibiotic resistance pattern of *E. coli*

Antibiotic resistant pattern	Number (%)
Amx-Cz	25 (41.7%)
Amx-E	1 (1.6%)
Amx-Nit	1(1.6%)
Amx-E-NA	1(1.6%)
Amx-E-Cz	3 (5%)
Amx-Cz-NA	3 (5%)
Amx-Cz-Nit	6 (10%)
Amx-E-Cz-NA	9 (15%)
Amx-E-Cz-Nit	6 (10%)
Amx-E-Cz-NA-Nit	5 (8.3%)
Total	60 (100%)

Table 3: Multiple antibiotic resistance index of *E. coli*

MAR Index	Frequency of MAR index *E. coli* (n=69)
0	0 (0.0%)
0.1	9 (13.04%)
0.2	27 (39.13%)
0.3	0 (0.0%)
0.4	13 (18.84%)
0.5	15 (21.73%)
0.6	0 (0.0%)
0.7	5 (7.24%)
0.8	0 (0.0%)
0.9	0 (0.0%)
1.0	0 (0.0%)

Among the ESBL screening drug, Cefotaxime displayed sensitivity and positive predictive value (PPV) of 100% and 80% respectively. Ceftazidime displayed the lowest sensitivity of 83.3% and a PPV of 62.5%. Despite of having 91.6% sensitivity, both Ceftriaxone and Cefpodoxime had lower PPV of 73.3% (Table 4). The Cefotaxime-clavulanate and Ceftazidime-clavulanate combined disk detected 12 *E. coli* to be ESBL confirmed isolates.

Table 4: Screening of *E. coli* isolates for ESBL production

Screening Agents	ESBL Screening		No. of confirmed ESBL producers	Sensitivity (%)	Positive predictive value (PPV)
Ceftriaxone (30µg)	Screen positives	15	11	91.6	73.3
	Screen negatives	54	1		
Cefpodoxime (10µg)	Screen positives	15	11	91.6	73.3
	Screen negatives	54	1		
Ceftazidime (30µg)	Screen positives	16	10	83.3	62.5
	Screen negatives	53	2		
Cefotaxime (30µg)	Screen positives	15	12	100	80
	Screen negatives	54	0		
Aztreonam (30µg)	Screen positives	18	10	83.3	55.5
	Screen negatives	51	2		

Discussion

In this study, the overall prevalence of *E. coli* was found to be 69 (65.7%) in total 105 isolates. Similar result was reported by Sharma *et al.*(2011) who found 67.5% *E. coli*. Most of the *E. coli* isolates showed the multidrug resistant (86.95%) in agreement with other studies (Bashar *et al.*, 2009; Moyo *et al.*, 2010; Hassan *et al.*, 2011; Sharma *et al.*, 2013). This study demonstrated 100% resistant of *E. coli* isolates to Amoxicillin which was similar to previously reported finding (Khadgi *et al.*, 2013). Resistance to penicillins may be determined by the organisms due to the production of penicillin destroying enzymes such as beta-lactamase (Forbes *et al.*, 2007).

With the highest sensitivity and PPV cefotaxime was found the most reliable agent for ESBL screening test. This result matches with other findings (Ho *et al.*, 2000; Poudyal *et al.*, 2011). TEM-1, TEM-2, and SHV-1 β-lactamases are the primary causes for resistance towards β-lactam antimicrobial agents among gram negative rods (Livermore, 1995). Two isolates screened as ESBL screen negatives by Ceftazidime, however, were found ESBL producers on confirmatory test. This suggests the possible production of CTX-M type ESBL by these isolates (Bush, 2008). CTX-M ESBLs differ from TEM and SHV types as they hydrolyse Cefotaxime and Ceftriaxone better compared to Ceftazidime (Lewis *et al.*, 2007). Out of 69 *E. coli* isolates, 12 (17.39%) confirmed to be ESBL positive. This result is in harmony with previous study (Chander et al., 2013).

Conclusion

This study reveals that *E. coli* is the most predominant pathogen in urinary tract infection accounting for 65.7 % in total positive isolates. ESBL producers were found higher in females 11 (91.6%) than males 1 (8.3%). The prevalence of MDR *E. coli* isolates was high i.e. 86.95%. Likewise, MAR index data revealed that isolates with lowest and highest MAR index are present in our surrounding that can pose health hazards.

The prevalence of ESBL producers was 17.39% among total *E. coli.* Ceftazidime had the lowest sensitivity detecting ESBL producers and can miss CTX-M producing bacteria thus signifying use of more than one screening agents and combination disk assay for more reliable detection of ESBL. Hence this kind of study aids in evaluating the exact cause and mechanism of rapid development of antibiotic resistance by bacteria.

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