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Research Article

Prevalence of Extended Spectrum Beta-Lactamase (ESBL) Producing Multidrug Resistance Gram-Negative Isolates Causing Urinary Tract Infection

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Abstract

Gram-negative bacteria are considered as the important cause to different types of infections related to urinary system. In hospitalized patients, the prevalence of multidrug resistance during the treatment of UTI is increasing tremendously. In addition, extended spectrum beta-lactamase (ESBL) producing gram-negative bacteria plays vital role in the increment of drug resistance cases. This study is intended to find out prevalence of ESBL producing gram-negative isolates from hospitalized UTI patients. A total of 1057 urine samples were analyzed. Different species of gram-negative bacteria were identified: *Escherichia coli* (71.3%); *Klebsiella pneumoniae* (9.8%); *Klebsiella oxytoca* (8.6%); *Proteus* spp. (4.6%); *Citrobacter fruendi* (2.3%), *Pseudomonas* spp. (1.7%); *Enterobacter* spp. (1.1%); and *Acinetobacter* spp. (0.6%). Similarly, 97 (55.7%) isolates were multidrug resistant in which *E. coli* accounts to be 67 (54.03%) and 31 (31.09%) bacterial isolates were detected as extended spectrum beta lactamase producers. Study on ESBL producers helps to control drug resistance cases and finding of this study on prevalence rate of ESBL producers from patients suffering from UTI aids in the selection of proper antibiotics for the treatment of the infection.

Keywords: *Extended Spectrum Beta Lactamase; Multidrug Resistance; Urinary Tract Infection*

Introduction

Millions of people each year are affected with different types of bacterial infection. Urinary tract infection (UTI) is very common diseases. UTI frequently occur in hospitalized patients compared to community people. The prevalence of the disease is more in developing countries due to poor hygiene, life style, malnutrition, and environmental condition [1]. Bacteria belonging to Enterobacteriaceae have been recognized as one of vital causes of nosocomial and community acquired infections. *Escherichia coli* is the most recognized causative agents for uncomplicated community acquired UTIs along with *Klebsiella* spp., other Enterobacteriaceae, *Staphylococcus saprophyticus* and *Enterococcus* [2].

Multidrug resistance is defined as insensitivity or resistance of microorganisms to the administrated antimicrobial medicines which are structurally unrelated and have different molecular targets despites earlier sensitivity to it [3]. Studies from WHO report have shown very high rate of resistance in bacteria such as *Escherichia coli* against antibiotics such as cephalosporin and fluoroquinolones, *Klebsiella pneumoniae* against cephalosporin and carbapenems [4]. β-Lactam antibiotics are commonly used to treat bacterial infections which

include penicillins; cephalosporins; carbapenems; and monobactams. Increased use of antibiotics, particularly the third generation of cephalosporins, has been associated with the emergence of β-Lactamases mediated bacterial resistance, which subsequently led to the development of Extended Spectrum Beta Lactamase (ESBL) producing bacteria. ESBLs are enzymes which contribute resistance to broad spectrum third generation cephalosporins antibiotics as well as monobactams (aztreonam) [5]. These enzymes hydrolyzed β-lactam ring of antibiotic, and as a result the antibiotic loose antimicrobial activity. ESBLs have been reported worldwide in many different genera of Enterobactericeae and *Pseudomonas aeruginosa* [6]. Bacteria which produce ESBL also show the resistant pattern with other different group of antibiotics. In addition to plasmid that encodes ESBLs, these bacteria also harbor other several resistance determinants. ESBL producers were isolated initially from person suffering from nosocomial infections. However, in recent days, many reports stated that these causative agents are also being isolated from community [7].

The first of the enzymes capable of hydrolyzing the newer β-lactams, SHV-2, was found in a single strain of *Klebsiella ozaenae* isolated in Germany. *E. coli* and *Klebsiella* spp. produces ESBLs enzymes frequently. Some other bacteria like *Enterobacter* spp.; *Proteus* spp.; *Salmonella* spp.; *Citrobacter* spp.; and *Serratiamarcescens* also produce ESBLs which contribute in multidrug resistance capacity of bacteria [8]. Multidrug resistant strains and ESBL producers are creating an alarming threat in the treatment of the infections. This study aims to find out multidrug resistance pattern and prevalence of ESBL producing gram-negative isolates from hospitalized UTI patients.

Materials and Methods

The study was conducted in Sahid Memorial Hospital, Kalanki, Nepal from August 2014 to January 2015.

Sample size: A total of 1057 urine samples were collected from patients clinically suspected of UTI and referred for urine culture by physicians.

Culture of the urine sample: Semi-quantitative culture technique was used to culture urine sample. A loopful of well mixed mid-stream urine sample was inoculated onto Blood Agar (BA) and MacConkey Agar (MA) using sterile calibrated loop. The culture plates were incubated at 37˚C for 1-2 days.

Identification of Isolates: For the identification of *Staphylococcus aureus*, catalase, oxidase, coagulase test, oxidative and fermentative test was performed. For the identification of gram negative bacteria, colony morphology, staining reactions and various biochemical properties were studied.

Antibiotic sensitivity test: The antibiotic sensitivity test was performed according to the Clinical and Laboratory Standards Institute (CLSI) recommended Kirby-bauer sensitivity test method (CLSI 2014). Antibiotics used were amikacin, ampicillin, nitrofurantoin, cotrimoxazole, cefotaxime, gentamycin, ofloxacin, ceftriaxone, ceftazidime and norfloxacin.

Criteria for multidrug resistance isolates: In this study, resistances to two or more than two antibiotics of different structural classes were considered to be multidrug resistance.

Screening and confirmatory test for ESBL production: For screening for ESBL production, azetronam (30 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg) and cefotaxime (30 mcg) were placed on the media inoculated with test inoculum and incubated for 24 hours. Bacterial isolates showing Aztreonam ≤ 27 mm, ceftriaxone ≤ 25 mm, ceftazidime ≤ 22 mm, cefotaxime ≤ 27 mm were suspected to be ESBL producer.

For the conformation of ESBL, combination disk method was used. The combination of ceftazidime and cefotaxime alone and in combination with calavunic acid (CA) (10 µg) were used. An increase ZOI of > 5 mm for either antimicrobial agent tested in combination with CA versus its zone when tested alone confirms ESBL positive.

Ethical Consideration: Verbal informed consent was taken from the patients attending the hospital suspected for UTI and the study was approved by Sahid Memorial Hospital, Kalanki, Nepal.

Results

Out of 1057 UTI suspected patients, 193 patients were suffering from UTI. Out of 193 patients, 27(13.96 %) were male and 166 (86.02 %) were female. Out of 193 bacterial isolates, 174 were gram negative bacteria. Predominantly, *E. coli* was common bacteria followed by: *Klebsiella pneumonia*; *Klebsiella oxytoca*; *Proteus* spp.; *Citrobacter fruendii*; *Pseudomonas* spp.; *Enterobacter* spp.; and *Acinetobacter* spp. Gram negative isolates were the predominant among the organisms causing UTI.

Isolated organism	Isolates (174)	Percentage (100%)
E. coli	124	71.3%
K. pneumoniae	17	9.8%
K. oxytoca	15	8.6%
Proteus spp.	8	4.6%
C. fruendii	4	2.3%
Pseudomonas spp.	3	1.7%
Enterobacter spp.	2	1.1%
Acinetobacter spp.	1	0.6%
Total	174	100%

Table 1: *Distribution pattern of Uro-pathogens.*

Among 124 isolates, *E. coli* 67 (54.03%) isolates were multidrug resistant. *K. pneuminae* showed 41.17% and *K. oxytoca* showed 73.33% of multidrug resistance. All the isolates of *Citrobacter fruend*ii were multidrug resistant.

	E. coli (N = 124)	*K. pneumonia* (N = 17)	*K. oxytoca* (N = 15)	*Proteus* spp. (N = 8)
Amikacin	90 (72.5%)	10 (58.8%)	7 (46.7%)	6 (75%)
Ampicillin	24 (19.3%)	3 (17.6%)	6 (40%)	2 (25%)
Nitrofurantoin	80 (64.5%)	13 (35.2%)	9 (60%)	4 (50%)
Cotrimoxazole	42 (33.8%)	6 (35.2%)	6 (40%)	4 (50%)
Cefotaxime	68 (54.8%)	12 (70.5%)	5 (33.3%)	1 (12.5%)
Gentamicin	105 (84.6%)	7 (41.1%)	7 (46.7%)	6 (75%)
Ofloxacin	59 (47.5%)	9 (52.9%)	8 (53.3%)	4 (50%)
Ceftriaxone	85 (68.5%)	10 (58.8%)	4 (26.7%)	5 (62.5%)
Ceftazidime	78 (62.9%)	14 (82.4%)	4 (26.7%)	5 (62.5%)
Norfloxacin	60 (48.3%)	10 (58.8%)	8 (53.3%)	1 (87.5%)

Table 2: *Antibiotic susceptibility pattern of isolated Uro-pathogens.*

Microorganism	MDR	Non-MDR
E. coli (N = 124)	67	57
K. pneumonia (N = 17)	7	10
K. oxytoca (N = 15)	11	4
Proteus spp. (N = 8)	6	2
Citrobacter fruendii (N = 4)	4	0
Pseudomonas spp. (N = 2)	1	2
Enterobacter spp. (N = 2)	0	2
Acinetobacter spp. (N = 1)	1	0
Total (174)	97	77

Table 3: *Frequency of MDR among gram negative isolates.*

Out of 97 Multi drug resistance isolates, 63 (64.9 %) isolated strains were ESBL screening test positive. 46 (68.7%) isolates of *E. coli* was ESBL screening positive.

Microorganism	ESBL Screening Test		ESBL Conformation	
	Positive	Negative	Positive	Negative
E. coli	46	21	19	27
K. pneumoniae	3	4	3	0
K. oxytoca	9	2	7	2
Proteus spp.	2	4	0	2
C. fruendii	1	3	0	1
Pseudomonas spp.	1	0	1	0
Acinetobacter spp.	1	0	1	0
Total	63	35	31	32

Table 4: *Frequency of ESBL among Gram negative MDR isolates causing UTI.*

Among the 193 bacterial isolates, 174 were Gram negative bacteria. Out of 174 Gram negative bacteria, 31(16.06%) of the bacterial isolates showed Extended spectrum beta-lactamase positive.

Figure 1: *Multidrug resistant E.coli isolate from urine(S.No:14).*
[Sesitive: OF: Ofloxacin; Resistant: LF: Levofloxacin, CX:Cloxacliin,AMX:Amoxycillin, COT: Cotrimoxazole]

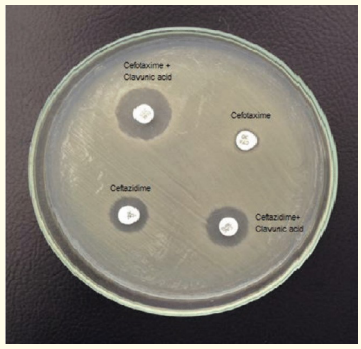

Figure 2: *Detection of ESBL producing E. coli by Combination disk method (S.N: 14).*

Discussion

Out of 1057 sample, 193 (18.26%) sample showed significant bacterial growth of the total bacterial isolates. Kattel., *et al*. (2008) [9] also observed similar low rate of growth positivity for UTI. The possible cause of low rate of growth positivity might be due to urine samples obtained from patients under treatment, infection due to slow growing organisms or due to those organisms that were not able to grow on the routine media we used. Among 193 samples, 27 were from male patients and 166 were from female patients. Female are more prone to UTI due to their anatomical structure. Gram negative bacteria were predominant causing UTI with the percentage of 90.1% while the Gram-positive bacteria were only 9.9%. In the study the prevalence of Gram negative bacteria was more than that of Gram positive bacteria causing urinary tract infection in human. A total of eight different species of bacteria were identified, in which *E. coli* accounts predominantly 64.2 % followed by: *S. aureus* (9.8 %); *K. pneumoniae* (8.8 %); *K. oxytoca* (7.7 %); *Proteus* spp. (4.1 %); *Citrobacter fruendii* (2.1 %); *Pseudomonas* spp. (1.6 %); *Enterobacter* spp. (1.1 %); and *Acinetobacter* spp. (0.6 %). *E. coli* was predominant and *Acinetobacter* being the least detected causative agents for UTI.

E. coli was predominant and *Acinetobacter* being the least isolated organism. Most of the isolates were the members of Enterobacteriaceace because they have the several factors for their attachment to the urothelium including adhesion, pilli, fimbrae, and P1 blood group genotype receptor [10]. In a similar study, 61.2 % *E. coli* was found to be the most common cause for UTI along with *Klebsiella* spp. (9.2 %); *P. aeruginosa* (7.1 %,); *E. faecalis* (6.1 %); *S. aureus* (6.1 %); and *C. freundii* (2.0 %); Coagulase negative *Staphylococcus* (2 %); *Morganella Morganii* (1%); and *Staphylococcus saprophyticus* (1 %) [11].

In both study the prevalence of *E. coli* was greater and nearly equal. *E. coli* contributes for more fifty percent of the isolated bacteria. The more prevalence of the *E. coli* could be attributed to the fact that it is commensal of the bowel and the route of transmission is by fecal contamination due to poor hygiene and the anatomical proximity to the genito urinary area in females [12]. In this study, the antibiotic susceptibility patterns of different bacterial isolates with different antibiotics were performed. Gentamicin, amikacin, and nitrofurantoin were shown to be most effective antibiotics among Gram negative isolates. 84.6 % of isolated *E. coli* was sensitive to the gentamycin, 72.5 % of the *E. coli* susceptible to amikacin and 64.5% of E. coli was sensitive to nitrofurantoin.

On the basis of this criterion, frequency of MDR was found to be 55.7%. This finding was somehow lower than some of the studies [9]. The cause of high MDR may be due to fact that the most cases of UTI are treated empirically especially in developing countries, where patients often cannot afford to consult a physician or have a laboratory tests. So, there may be over cases of microorganisms that are not responded to treatment [13]. Among 124, 54.03% of *E. coli* showed the pattern of multidrug resistance. Among 17 isolates of *K. pneu-*

moniae isolated from urine, 7 (41.17%) of *K. pneumoniae* were multi drug resistance. Among 15 isolates of *Klebsiella oxytoca*, 11 (73.33%) of *Klebsiella oxytoca* were multidrug resistance. Among 8 *Proteus* spp. isolated from urine, 75 % were multidrug resistant strains. Among 3 isolates of *Pseudomonas* spp., 1 (33.33%) isolate was multidrug resistance. All 4 isolates of *Citrobacter fruendii* were multidrug resistance. A study conducted by Manandhar., *et al*. (2006) [11] mentioned that cases of multidrug resistance in *E. coli* were accounted to be 61.7 % and *K. oxytoca* and *K. pneumoniae* was found to be 40% and 75% respectively. In this study, the prevalence of MDR contributed by *E. coli* and K. pneumoniae were greater. This indicates that the emergence of MDR strains is common in the UTI patients visiting Sahid Memorial Hospital and this may be due to empirical treatment of UTI. Nepal, being a developing country has less facility for the healthcare. There are huge trends of taking antibiotics without proper laboratory investigation of diseases. Similarly, inappropriate dosage of drug prescribed by medical personnel also seems to contribute for prevalence of multidrug resistance in Nepal.

The high level of drug resistance seen among *E. coli* infected patients which may be due to production of beta-lactamases enzyme. This enzyme break the beta-lactam ring inactivating the antibiotic. The classical TEM-1, TEM-2, and SHV-1 enzymes are the predominant plasmid-mediated beta-lactamases of most of the gram-negative rods [14]. Mutations on the target sites i.e. gyrA, which is a gyrase subunit gene, and *parC*, which encodes a topoisomerase subunit, confer resistance to fluoroquinolones [15]. For all the gram-negative isolates, which showed multidrug resistance (MDR) property, screening of extended spectrum beta lactamase (ESBL) was carried out for the preliminary detection of ESBL producer. Among 97 MDR Gram negative isolates, 63 (64.9%) showed the ESBL screening positive result.

The ESBL mutant which are derived from older, broad spectrum beta lactamase (eg. TEM-1, TEM-2, SHV-1) mediate resistance to extended spectrum third generation antibiotic cephalosporins: ceftazidime, cefotaxime, ceftriaxone. These mutants are specific to third and fourth generation cephalosporins but not to cephamycins (cefoxitin and cefotetan) or carbapenams (meropenam or imipenam) [16].

Excessive use of broad spectrum antibiotics in the hospitals may be the reason for higher production of ESBL by bacteria. About 15.32 % of the *E. coli*; 46.66% of the *K. oxytoca*; and 17.64% of the *K. pneumonia* was ESBL producer. In a similar study carried by Manandhar., *et al*. (2006) [11], 26.7 % of *E. coli* and 50% of *K. pneumoniae* were found to produce ESBL. Similarly, a study of *Pseudomonas aeruginosa* isolated from various clinical specimens in turkey, 36% of isolates were resistant to more than one group of antibiotics [17].

In contrast, a study conducted in 11,865 *E. coli* urinary isolates obtained from community and hospitalized patients in East Londen, high rates of resistance to ampicillin (55%) and trimethoprism (40%), often in combination were observed in both sets of isolates. Inspite of isolates exhibiting resistance to multiple drugs were rare, resistance to cefpodoxime, indicative of extended spectrum β-lactamase production, was found in 5.7 % of community and 21.6 % of nosocomial isolates [18].

Resistance to antimicrobial drugs causes increase in morbidity and mortality due to infectious diseases. Antibiotic resistance is a worldwide problem. In recent days, these issues are generally considered as public health problem and have significant effects in health. The problem of the bacterial resistance to antimicrobial drugs is more troublesome to developing countries like Nepal.

Conclusion

The major predominant causative agent of urinary tract infection was *E. col*i followed by *K. pnemoniae*. The prevalence of urinary tract infection (UTI) among the patients suspected with UTI was found to be 18.3%. The frequency of MDR isolates causing UTI was found to be 55.7%. ESBL producers among the total bacterial isolates from urinary tract infection were found to be 16.06%. Gram negative isolates from urinary tract infection were found to be most sensitive to gentamicin followed by amikacin, ceftazidime, nitrofurantoin. Resistance to the ampicillin, cotrimoxazole were shown by most of the bacterial isolates causing urinary tract infections.

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