Phenotypic Characterization of Multidrug-resistant Escherichia Coli with Special Reference to Extended-spectrum-beta-lactamases and Metallo-beta-lactamases in a Tertiary Care Center

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ABSTRACT

Introduction: The increasing reports on extended-spectrum-beta-lactamase and metallo-beta-lactamase producing *Escherichia* coli have addressed a potential threat to global health since it is found to be highly resistance to most of the currently available antibiotics including carbapenems. The present study was aimed to determine the antibiogram of extended-spectrum-beta-lactamase and metallo-beta-lactamase producing MDR *E. coli* isolates from various clinical samples.

Methods: This was a cross-sectional study conducted over a period of seven months (December 2013 to July 2014) at bacteriology laboratory of Tribhuvan University Teaching Hospital. A total of 250 clinical specimens (urine, pus, sputum, blood, body fluid, bile, tissue and central venous pressure line tip) were processed from inpatients, with multidrug-resistant (MDR) *Escherichia coli* infections. Standard microbiological techniques were used for isolation and identification of the isolates. The presence of extended-spectrum-beta-lactamase was detected by phenotypic confirmatory test recommended by Clinical and Laboratory Standards Institute and imipenem (IMP) / EDTA combined disc method was performed to detect metallo-beta-lactamase mediated resistance mechanism.

Results: We found high level of beta lactamase mediated resistance mechanism as part of multidrug resistance. Among 250 MDR isolates, 60% isolates were extended-spectrum-beta-lactamase producers and 17.2% isolates were metallo-beta-lactamase producers. Co-existence of extended-spectrum-beta-lactamase and metallo-beta-lactamase identified in 6.8% isolates.

Conclusions: Beta-lactamase mediated resistance mechanisms are accounting very high in the multidrug resistant isolates of *E. coli.* Therefore, early detection of beta lactamase mediated resistant strains and their current antibiotic susceptibility pattern is necessary to avoid treatment failure and prevent the spread of MDR.

Keywords: *e. coli; extended-spectrum-β-lactamase; metallo-β-lactamase; multidrug-resistance.*

INTRODUCTION

The production of beta-lactamases (β-lactamases) is the most common mechanism responsible for resistance to β-lactams among clinical isolates of Enterobacteriaceae family.[1] The β-lactamases receiving the most attention are the extended-spectrum-beta-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases and carbapenemases because of rapid global dissemination of these enzymes.[1,2] ESBLs confer bacterial resistance to all β-lactams except carbapenems and cephamycins, which are inhibited by β-lactamase inhibitors such as clavulanic acid.[3] Carbapenemases consist of serine-β-lactamases (KPC, OXA, GES, etc.) and Metallo-β-lactamases (MBLs) which are associated with resistance to aminoglycosides and fluoroquinolones.[4]

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E.coli has been reported as opportunistic, worrisome, nosocomial and community-associated pathogen and the most frequent isolate in various clinical specimens.[5] *E.coli* is a major concern in medical community because of worldwide emergence of MDR strains mediated by ESBL and MBL enzymes.[3,6,7]

Little information is currently available regarding ESBL and MBL producing *E.coli* in Nepal. Keeping in view the above background, this cross-sectional study was conducted to provide information on antibiotic susceptibility with special reference to ESBL and MBL in MDR *E.coli* isolates from hospitalized patients.

METHODS

A cross-sectional study was conducted at the bacteriology laboratory of Tribhuvan University Teaching Hospital (TUTH), Kathmandu, over a period of seven months from 12th December 2013 to 12th July 2014. This study was approved by Institutional Review Board of Institute of Medicine. A total of 250 consecutive non-repetitive multidrug-resistant *Escherichia coli* (MDR *E. coli*) were isolated from various clinical samples such as urine (n = 127), pus (n = 65), sputum (n = 37), blood (n = 9), body fluid (n = 4), bile (n = 5), tissue (n = 2) central venous pressure (CVP) line (n = 1) which were received from hospitalized patients. The isolation and identification of *Escherichia coli* were performed following standard microbiological techniques as described by American Society of Microbiology (ASM).[8] Data were analyzed using Microsoft Excel 2007.

Antimicrobial susceptibility testing

Antibiotic susceptibilities were determined by Kirby-Bauer disk diffusion method and the results were interpreted according to the guidelines of the Clinical Laboratory Standard Institute (CLSI).[9] The antibiotic discs used were amikacin (30 μg), amoxycillin (10 μg), amoxycillin/clavulanic acid (20/10 μg), cefepime (30 μg), cefoperazone/sulbactam (75/30 μg) ceftazidime (30 μg), cefoxitin (30 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), colistin sulphate (10 μg), cotrimoxazole (1.25/23.75 μg), doxycycline (30 μg), gentamicin (10 μg), imipenem(10 μg), meropenem (10 μg), nitrofurantoin (300 μg), ofloxacin (5 μg), piperacillin/tazobactam (100/10 μg), polymyxin-B (300 units) and tigecycline (5 μg).

Definition of MDR

MDR *E.coli* were defined as the isolates of *E.coli* resistant to at least three classes of antimicrobial agents-all penicillins and cephalosporins (including inhibitor combinations), aminoglycosides, cephamycins, fluoroquinolones, folate pathway inhibitors, glycylcyclines, phenicol, polymyxins and tetracyclines.[10]

Tests for ESBL-production

All of the 250 isolates were screened for ESBL production by CLSI phenotypic confirmatory test of combined disc assay method.[9] One disc of ceftazidime (30 μg) alone and one in combination with clavulanic acid (30 μg /10 μg) were placed at a distance of 20 mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight at 37°C. The ESBL-producing strains showed a variation greater than 5mm between the inhibition zones around cefotaxime or ceftazidime discs alone in comparison with the inhibition zone around cefotaxime/clavulanic acid or ceftazidime/clavulanic acid discs. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative control strains respectively.

Tests for MBL-production

Screening test: The isolates were subjected for MBL detection when the zone of inhibition (ZOI) for ceftazidime (CAZ) (30 μg) was < 18 mm. The sensitivity or resistivity pattern to imipenem (IPM) (10mg) and or meropenem (MEM) (10 μg) were not considered for MBL detection as bacteria might harbour "hidden MBL" and if only the carbapenem resistant phenotypes were considered, then such hidden MBL carrying isolates would be missed.

MBL confirmation by combination disk method: All 250 isolates were phenotypically confirmed for metallo-β -lactamase production as described by Franklin et al.[11] Briefly, two imipenem (IPM) disks (10 μg), one containing 10 μl of 0.1 M (292 μg) anhydrous EDTA (Sigma Chemicals, St. Louis, MO), were placed 25 mm apart (center to center). An increase in zone diameter of >4 mm around the IPM/EDTA disk compared to that of the IPM disk alone was considered positive for an MBL. For MBL test standardization, *Pseudomonas aeruginosa* ATCC 27853 was used as a negative control strain and *Pseudomonas aeruginosa* PA 105663 was used as a positive control.

RESULTS

Two hundred and fifty specimens with MDR *E. coli* infection were included in this study. These included (n = 127, 50.8%) urine, (n = 65, 26 %) pus, (n = 36, 14.4%) sputum, (n = 9, 3.6%) blood, 5 (2.0%) bile, (n = 4,1.6%) body fluid, (n = 2, 0.8%) tissue, (n = 1, 0.4%) endotracheal secretion and (n = 1, 0.4%) CVP line.

The highest number of MDR *E. coli* (n = 141, 56.4%) was isolated from surgical wards (Table 1).

Table 1. Prevalence of MDR *E. coli* in different wards (n = 250).

Wards	No (%)
Surgical	141 (56.4)
ICU	21 (8.4)
Medical	18 (7.2)
Neuro	18 (7.2)
Pediatric	14 (5.6)
Post-operative	14 (5.6)
Maternity	7 (2.8)
Nephro	7 (2.8)
Orthopedics	5 (2.0)
Pediatric ICU	2 (0.8)
Burn	1 (0.4)
Eye	1 (0.4)
Surgical ICU	1 (0.4)

Antibiogram of MDR E.coli

Out of 21 antibiotics used for antibiotic susceptibility test, colistin sulphate, polymyxin-B and tigecycline were 100% effective followed by imipenem (n = 204, 81.6%), chloramphenicol (n = 90, 73.6%), amikacin (n = 170, 68%), meropenem (n = 149, 59.6%) and piperacillin-tazobactam (n = 146, 58.4%). All isolates were 100% resistance to amoxycillin, cefotaxime, ceftazidime, and ceftriaxone (Table 2).

Distribution of ESBL and MBL in MDR E .coli

Out of 250 *E. coli* strains studied, (n = 239, 95.6%) strains produced any of the 2 types of β – lactamases i.e. ESBL and MBL, either alone or co-producer (Figure 2). In our study, (n = 15, 6.2%) *E. coli* strains were positive for both types of β–lactamases i.e. ESBL and MBL in combination.

Pattern of ESBL and MBL producers E. coli in different clinical specimens

Out of total 150 ESBL positive *E. coli* strains, maximum (n = 91, 60.6%) strains were isolated from urine followed by (n = 31, 20.6%) strains from pus, and (n = 18, 12.0%) from sputum. Out of 43 MBL producers, (n = 17, 39.5%) were isolated from urine and (n = 14, 32.5%) were isolated from pus. No MBL producing *E. coli* strain was isolated from body fluids and tissue (Table 3).

Distribution of ESBL and MBL- producers in different wards

ESBL and MBL producing MDR *E. coli* were the most common in surgical wards. Out of 250 MDR *E. coli* isolates, (n = 80, 53.3%) ESBL-producers and (n = 26, 60.4%) MBL-producers were isolated from surgical wards (Table 4).

Table 2. Antibiogram of MDR E.coli (n = 250).

Antibiotics	Sensitive	Resistant
	No (%)	No (%)
Colistin sulphate	250 (100.0)	0 (0.0)
Polymyxin-B	250 (100.0)	0 (0.0)
Tigecycline	250 (100.0)	0 (0.0)
Imipenem	204 (81.6)	46 (18.4)
Chloramphenicol**	90 (73.8)	33 (26.2)
Amikacin	170 (68.0)	80 (32.0)
Meropenem	149 (59.6)	101 (40.4)
Nitrofurantoin*	75 (59.0)	28 (41.0)
Piperacillin /tazobactam	146 (58.4)	104 (41.6)
Gentamicin	108 (43.2)	142 (56.8)
Cefoperazone / sulbactam	102 (40.8)	148 (59.2)
Doxycycline	63 (25.2)	187 (74.8)
Cefoxitin	74 (29.6)	176 (70.4)
Amoxycillin/ clavulanic acid	64 (25.6)	186 (74.4)
Cefepime	23 (9.2)	227 (90.8)
Cotrimoxazole	23 (9.2)	227 (90.8)
Ciprofloxacin	10 (4.0)	240 (96.0)
Ofloxacin	10 (4.0)	240 (96.0)
Ceftazidime	1 (0.4)	249 (99.6)
Cefotaxime	0 (0.0)	250 (100.0)
Ceftriaxone	0 (0.0)	250 (100.0)
Amoxycillin	0 (0.0)	250 (100.0)

** Except for urinary isolates

* Only for urinary isolates

Table 3. Isolation of ESBL and MBL producers E.coli from different clinical specimens.

Specimen	ESBL	MBL	ESBL + MBL
	No (%)	No. (%)	No. (%)
Urine	91 (60.6)	18 (41.8)	9 (60.0)
Pus	31 (20.7)	14 (32.5)	2 (13.3)
Sputum	18 (12.0)	8 (18.6)	4 (26.7)
Blood	4 (2.7)	2 (4.6)	0 (0.0)
Body fluid	3 (2.0)	0 (0.0)	0 (0.0)
Tissue	2 (1.3)	0 (0.0)	0 (0.0)
Bile	1 (0.7)	1 (1.5)	0 (0.0)
Total	150 (100.0)	43 (100.0)	15 (100.0)

Antibiogram of ESBL -producer MDR E. coli

All ESBL-producers were highly susceptible to imipenem (n = 135, 90.0%) and meropenem (n = 120, 80.0%) followed by piperacillin/tazobactam (n = 119, 79.0%), chloramphenicol (n = 46, 78.0%) and amikacin (n = 117, 78.0%) but most of the isolates were resistance to third generation cephalosporins and non-β-lactam antibiotics (Table 5).

Table 4. Distribution of ESBL and MBL- producer E. coli strains among different wards (n = 250).

Wards	ESBL	MBL	ESBL + MBL
	No. (%)	No. (%)	No. (%)
Surgical	80 (53.3)	26 (60.4)	9 (60.0)
Medical	13 (8.7)	1 (2.3)	1 (6.7)
Neuro	12 (8.0)	5 (11.8)	1 (6.7)
ICU	11 (7.3)	2 (4.6)	1 (6.7)
Paediatric	9 (6.0)	2 (4.6)	2 (13.3)
POW	7 (5.0)	3 (7.0)	0 (0.0)
Maternity	6 (4.0)	0 (0.0)	0 (0.0)
Nephro	5 (3.3)	0 (0.0)	0 (0.0)
Orthopedics	3 (2.0)	1 (2.3)	0 (0.0)
NICU	1 (0.7)	2 (4.7)	0 (0.0)
PICU	1 (0.7)	1 (2.3)	1 (0.0)
Burn	1 (0.7)	0 (0.0)	0 (0.0)
Eye	1 (0.7)	0 (0.0)	0 (0.0)
Total	150 (100.0)	43 (100.0)	15 (100.0)

Table 5. Antibiogram of ESBL producer MDR E.coli (n = 150).

Antibiotics	Sensitive	Resistant
	No (%)	No (%)
Imipenem	135 (90.0)	15 (10.0)
Meropenem	120 (80.0)	30 (20.0)
Piperacillin + Tazobactam	119 (79.3)	31 (20.7)
Chloramphenicol**	46 (78.0)	13 (22.0)
Amikacin	117 (78.0)	33 (22.0)
Cefoperazone/ sulbactam	84 (56.0)	16 (44.0)
Cefoxitin	73 (48.7)	77 (51.3)
Gentamicin	72 (48.0)	78 (52.0)
Doxycycline	43 (28.6)	107 (71.3)
Nitrofurantoin*	51 (56.0)	40 (44.0)
Amoxycillin/ clavulanic acid	49 (32.7)	101 (67.3)
Cefepime	17 (11.3)	133 (88.7)
Cotrimoxazole	12 (8.0)	142 (92.0)
Ciprofloxacin	10 (6.7)	140 (93.3)
Ofloxacin	10 (6.7)	140 (93.3)
Ceftazidime	1 (0.4)	149 (99.6)
Cefotaxime	0 (0.0)	150 (100.0)
Ceftriaxone	0 (0.0)	150 (100.0)
Amoxycillin	0 (0.0)	150 (100.0)

** Except urinary isolates

* Only for urinary isolates

Antibiogram of MBL-producer MDR E. coli

Antibiotic susceptibility tests of MBL producers revealed totally resistance to all penicillins and cephalosporins (including inhibitor combinations) and carbapenems. The isolates showed high percentage of resistance to most antibiotics tested: ciprofloxacin (n = 42, 97.7%), ofloxacin (n = 42, 97.7%), gentamicin (n = 41, 95.3%), cotrimoxazole (n = 41, 95.3%), piperacillin/ tazobactam (n = 41, 95.3%), Amikacin (n = 38, 88.3%), nitrofurantoin (n = 11, 83.7%) and doxycycline (n = 34, 79.1%). Polymyxins and tigecycline were the most effective antibiotics (n = 43, 100.0%) against MBL-producer MDR *E. coli* followed by chloramphenicol (n = 13, 52.0%).

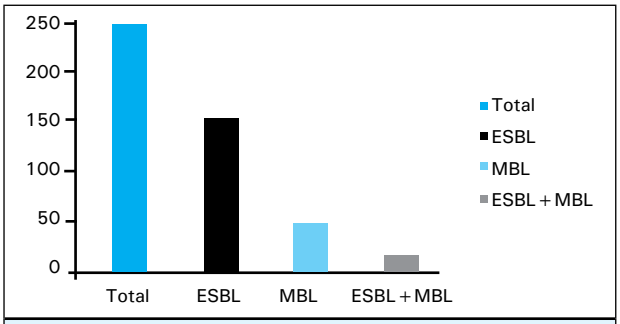

Figure 1. Distribution of ESBL and MBL in MDR *E .coli* (n = 250).

Antibiogram of ESBL and MBL co-producer MDR E. coli

All the ESBL and MBL co-producer isolates were highly sensitive (n = 15, 100.0%) to polymyxins and tigecycline but completely resistant to all penicillins and cephalosporins (including inhibitor combinations), aminoglycosides, cephamycins, fluoroquinolones, folate pathway inhibitors and nitrofurantoin.

DISCUSSION

ESBL and MBL enzymes are of increasing clinical concern. ESBLs are most commonly produced by *Escherichia coli* and *Klebsiella* spp. but may also be present in other gram negative bacteria. Many MDR bacteria produce multiple β-lactamases including combinations of these different enzymes. With the increasing number of MBL and KPC producing bacteria and ESBL and AmpC producing bacteria associated with porin loss and efflux mechanisms, there has been an increasing resistance to carbapenems.[4] Prolonged antibiotic exposure, overstay in hospitals, severe illness, unprecedented use of third generation cephalosporin, and increased use of intravenous devices or catheters are important risk factors for infection with MDR *E. coli.*[12]

The current study demonstrated that (n = 127, 50.8%) of MDR *E. coli* were isolated from urine samples and (n = 141, 56.4%) from surgical wards. With regard to urinary tract infection among hospitalized patients, many researchers indicated its incidence as: 31%-47%.[13-15] In the present study, most of the patients in surgical wards have indwelling urinary catheter. The indwelling urinary catheter as an invasive device has a significant association with hospital acquired urinary tract infections for it provides either a portal of entry for microorganism or a place for colonization of microorganisms.[16]

For all of MDR *E.coli* isolates, colistin, polymyxin B and tigecycline had the excellent activity followed by chloramphenicol, Piperacillin/tazobactam, amikacin and carbapenems. The resistance of *E. coli* isolates towards the third generation and fourth generation cephalosporins– cefotaxime and cefepime could be attributed to ESBL or some other relevant underlying mechanisms. Results of our study have shown that infection with ESBL producing MDR *E. coli* was (n = 150, 60.0%) in our setting which was alarmingly high. Others have reported 50-70% prevalence of ESBL producing among MDR *E. coli*.[17,18] ESBL production varies from hospital to hospital because of variation in selection of type of antibiotics. The selective pressures which are generated by the indiscriminate use of the beta-lactam antibiotics have led to the selection of a variety of mutated forms of β-lactamases such as ESBLs.[19]

In this study (n = 43, 17.0%) isolates were MBL producers. Various authors have reported 2.9%-25% MBL producing *E.coli* strains from hospitalized patients.[20-22] Our findings were in concordance with the studies which were done by Bora et al[23] who reported 18.9% and Bandekar et al,[24] who reported 15.7% MBL producers. MBL producing organisms were isolated mainly from surgical wards (n = 26, 60.4%). Indwelling medical devices are commonly used in these wards, which play a key role in the spread of infective agents. In any hospital setting, carbapenems are used as the last resort for treatment of MDR gram-negative bacterial infection. Antibiotic overuse is an important contributor for the emergence and spread of resistance; association between carbapenem consumption and resistance has been previously documented.[25] However, since last 15 years, acquired resistance which is mainly mediated by MBLs to these life saving antimicrobials has been increasingly reported worldwide including Nepal not only in *Pseudomonas* and *Acinetobacter* spp, but also among members of *Enterobacteriaceae*.[26,27]

Analysis of antimicrobial susceptibility pattern of ESBL producing *E. coli* isolates demonstrated high susceptibility rates towards imipenem(n = 135, 90.0%) and meropenem (n = 120, 80.0%) followed by piperacillin/tazobactam (n = 119, 79.0%), amikacin (n = 117, 78.0%) and chloramphenicol (n = 46, 78.0%). Similar susceptibility patterns were observed in studies conducted in Nepal and India.[28-30] MBL producing bacterial isolates can confer resistance to carbapenems and all beta-lactam agents except aztreonam although coexistence of other resistance mechanisms such as AmpC type beta-lactamases or ESBLs render them resistant to aztreonam.[31] We observed all MBL producer *E.coli* were resistant to imipenem and meropenem. These isolates also demonstrated a high level of resistance to amoxycillin, the third and fourth generation cephalosporins, amikacin and gentamicin as well as to the beta-lactam/beta-lactamase inhibitor combination tested in the study. These findings are similar with other reports.[23,27] Present study identified ESBL and MBL co-producers in (n = 15, 6.2%) isolates. An increased rate of occurrence of ESBL and MBL co-producers (8.7 %) was also observed among nosocomial isolates of *E.coli* in a recent report from India.[32] This study has demonstrated a very high level of resistance to the most of antibiotics tested in ESBL and MBL co-producer *E.coli.* Only polymyxins and tigecycline have potent activity against these isolates. Although the isolates were uniformly susceptible to polymyxin B and tigecycline in vitro, outcomes for infected patients treated with these agents remain unknown.[33] The coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges because the treatment options are fast running out. They are of significant concern because they create therapeutic dilemma, cause treatment failures and are increasing in occurrence worldwide. It might be undertaken that in the absence of novel agents in the near future, the spread of ESBL and MBL co-producers may lead to therapeutic dead ends.

CONCLUSIONS

Of particular concern are our results showing frequent carbapenem resistance among *E. coli* isolates, as well as the high rates of resistance to non-beta-lactam agents. This report underlined a real threat from the emergence of extreme drug-resistant and pan drug-resistant bacteria in near future. The spread of ESBL and MBL producing bacteria has been noticeably rapid worldwide including Nepal, indicating that continuous monitoring systems and effective infection control measures are absolutely required. Therapeutic options for infections due to ESBL and MBL producers have also become increasingly limited. Therefore, a better understanding of β-lactamase mediated resistance mechanisms is critical for optimizing therapy. In view of the exhaustion of available therapeutic options, investment in infection control resources and optimal antibiotic use, along with harmonized efforts from all concerned authorities is urgently required.

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