Journal of Microbiology and Infectious Diseases / JMID
2013; 3 (4): 150-156
doi: 10.5799/ahinjs.02.2013.04.0100

RESEARCH ARTICLE

Expression of ESBL, MBL and AmpC β lactamases by extra intestinal *Escherichia coli* isolates: correlation with treatment and clinical outcome

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ABSTRACT

Objective: We investigated the expression of Extended Spectrum β-Lactamases (ESBLs), AmpC β lactamases and Carbapenemases in extraintestinal pathogenic *Escherichia coli* (ExPEC) isolates and correlated with treatment and outcome of the patients.

Methods: Three hundred ExPEC infected patients were included in the study. Demographic data, antibiogram, treatment and outcome were collected. Production of ESBLs was detected by combination disk method; AmpC was detected by AmpC disk test. Carbapenemase production was detected by disk diffusion and confirmed by modified Hodge test. Identification of metallo- β-lactamase (MBL) activity was performed by the carbapenem-EDTA combined disk method and MBL E-test.

Results: Out of 300 *E. coli* isolates, 212 (71%) were ESBL producers. AmpC β lactamase production was seen in 95 (32%) isolates; 16 (17%) isolates were pure AmpC producers whereas 79 (83%) were ESBL co-producers. Twenty nine (9.5%) isolates were carbapenemase producers of which 15 (5%) were MBL producers. For treatment, most widely prescribed antibiotics were β-lactam+β-lactamase inhibitor combinations (39%). Sixty seven percent patients improved; relapse/re-infection was seen in 18% of patients and 11% patients expired. Increased mortality was seen in patients with blood stream infection and more number of relapses was seen in urinary tract infection.

Conclusion: ExPEC producing ESBL or AmpC along with carbapenemases are particularly challenging for clinicians and are a major threat worldwide. Early use of appropriate antibiotics like β-lactam+β-lactamase inhibitor combinations will probably reduce complications in these patients.

Key words: AmpC, ExPEC, ESBL, MBL, *J Microbiol Infect Dis 2013;3(4): 150-156*

Ekstra intestinal Escherichia coli izolatlarından GSBL, MBL ve AmpC β laktamazların salınımı: Tedavi ve klinik sonuçlarla uyumu

ÖZET

Amaç: Ekstra intestinal patojenik *Escherichia coli* (EİPEC) izolatlarından salınan GSBL, AmpC β laktamazlar ve Karbapenemazların salınımını ve bu hastaların tedavisi ve klinik gidişle ilişkisini araştırdık.

Yöntemler: Çalışmaya 300 EİPEC ile enfekte hasta alındı. Demografik veriler, antibiyogram, tedavisi ve sonuçları toplandı. GSBL üretimi kombine disk metodu ve AmpC üretimi AmpC-disk testi ile tespit edildi. Karbapenemaz salınımına modifiye Hodge testi ile doğrulanmış disk diffüzyonla bakıldı. Metallo-β-laktamaz (MBL) aktivitesi karbapenem-EDTA kombine disk metodu ve MBL E-test ile çalışıldı.

Bulgular: Üç yüz *E. coli* izolatının 212'si (% 71) GSBL pozitif idi. AmpC β laktamaz üretimi 95 (% 32) görüldü; izolatların 16'sı (% 17) saf AmpC üretirken, 79'u (% 83) GSBL ile beraberdi. Karbapenemaz üreten yirmi dokuz (% 9,5) izolatın 15'i (% 5) MBL üretiyordu. Tedavi için, en yaygın olarak reçete edilen antibiyotikler β-laktam + β-laktamaz inhibitör kombinasyonları (% 39) idi. Hastaların % 67'i iyileşti; nüks/yeni enfeksiyon hastaların % 18'inde görülürken % 11 hasta da öldü. Kan dolaşımı enfeksiyonu olan ve sık nüks eden idrar yolu enfeksiyonlarında mortalite daha yüksekti.

Sonuç: Karbepenamazlarla birlikte GSBL veya AMPC üreten EİPEC özellikle klinisyenler için dünya çapında büyük bir tehdit oluşturmaktadır β laktam + β laktamaz inhibitör kombinasyonu gibi uygun antibiyotiklerin erken kullanımı muhtemelen bu hastalarda komplikasyonları azaltacaktır.

Anahtar kelimeler: AmpC, EİPEC, GSBL, MBL

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Received: 30.11.2012, Accepted: 07.08.2013
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INTRODUCTION

Infection with extraintestinal pathogenic *Escherichia coli* (ExPEC) is an important public health problem worldwide. They are responsible for urinary tract, intra-abdominal and soft tissue infections, meningitis, pneumonia and osteomyelitis and are often associated with bacteremia.[1] The prevalence of multidrug-resistant ExPEC has increased progressively over the past few years, and infections with bacterial strains producing carbapenemases, AmpC beta lactamases and/or extended spectrum beta-lactamases are of particular concern.[2,3] For several years carbapenemases were highly effective against bacteria that exhibited resistance to extended spectrum cephalosporins (e.g. Ceftazidime and Cefepime), including ESBL and AmpC producers. However, as carbapenemases began to emerge worldwide, the effectiveness of this last-line antibiotic class was challenged.[4]

There is insufficient data regarding expression of ESBL, AmpC and metallo-β-lactamases (MBL) by *E. coli* strains causing extraintestinal infections in India. Hence, the present study was undertaken to find out the prevalence of ESBL, AmpC and MBL production among ExPEC isolates in a tertiary care hospital and to co-relate such infections with treatment and clinical outcome.

METHODS

Participants and clinical isolates: The study was conducted during the period from August 2010 to January 2012, from patients of the tertiary care hospitals attached to Kasturba Medical College, Mangalore, India, after obtaining permission from the institutional ethical committee. Sample size was determined with 55% confidence level and 90% power according to earlier study.5 Three hundred strains of *E. coli* were isolated from specimen such as urine, blood, wound swab, pus, CSF, ascites fluid and intravascular devices from the study population. Study population included patients of all age groups whose clinical samples grew *E. coli* and excluded subjects who had received antimicrobial drugs during past one month, who had asymptomatic UTI, polymicrobial infections and those who were discharged without treatment. Details of antibiotics used and clinical outcome of patients were collected. Samples were processed immediately using standard procedures. Isolates were identified based on colony morphology on Blood agar, MacConkey's agar, Gram staining and by standard biochemical tests.5 Blood isolates were identified using automated biochemical system Vitek 2 (bioMerieux).

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was done by the modified Kirby-Bauer disk diffusion method in accordance with CLSI guidelines.[6] The antibiotic disks (HiMedia, Mumbai, India) used were Ampicillin (10 µg), Piperacillin (10 µg), Piperacillin/Tazobactam (100/10 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg), Norfloxacin (10 µg), Amikacin (30 µg), Gentamicin (10 µg), Cotrimoxazole (1.25/23.75 µg), Cefoperazone + Sulbactam (75/30 µg), Imepenem (IPM; 10 µg), Meropenem (MRP; 10 µg) and Etrapenem (ETP; 10 µg).

Screening for ESBL production

Isolates which were resistant to third generation cephalosporins were tested for ESBL production by combination disk method using cefotaxime (30 µg), cefotaxime/clavulanic acid (10 µg), ceftazidime (30 µg) and ceftazidime/clavulanic acid (10 µg). A ≥5mm increase in diameter of inhibition zone of cephalosporin+clavulanate disc when compared to cephalosporin disc alone was interpreted as evidence of ESBL production.[6]

Detection of AmpC production

Isolates were tested for AmpC enzyme production by AmpC disk test.[7] Briefly, a suspension of ATCC *E. coli* 25922 standardized to 0.5 McFarland was inoculated on the surface of Mueller-Hinton agar (MHA) plate. A 30 µg cefoxitin disk were placed on the inoculated surface of the agar.

Detection of carbapenemase production

Plates of MHA were inoculated with standardized suspensions of the test strains. A sat of discs of IPM, MRP and ETP (10 µg each) were applied to the surface of the agar, plates were incubated overnight at 35°C aerobically, and diameters of zone of inhibition (≥23 mm indicated sensitivity, 20 to 22 mm indicated intermediate resistance and ≤19 mm indicated resistance) were recorded. Carbapenemase production was further confirmed by modified Hodge test (MHT).[6]

Detection of metallo- β- lactamase producers

Identification of MBL activity was performed by two methods: a carbapenem-EDTA combined disk method and MBL E-test (HiMedia, India).[8] A known MBL producing isolate was used as positive control for all tests. Combined disk test: The IPM-EDTA combined disk method was performed as described previously.8 MBL E-test (IPM-EDTA E-test, HiMedia) was used to detect MBL production and MIC of

IPM to the test isolates and was performed by E-test according to the recommendations of the manufacturer.[8]

Imipenem MIC

E-Strips were used for determination MIC of Imipenem.[8] Briefly, test isolates were inoculated in a Mueller Hinton Agar plate. Strips were placed at a desired position on agar plate swabbed with test culture. The plates were incubated overnight at 37°C aerobically. Interpretation of MIC values: <8 µg/ml=Sensitive; 8-16 µg/ml=Intermediate resistance; >16 µg/ml=Resistant.

Statistical analysis

Chi-square test was used to find association between ESBL, AmpC and carbapenemase producers. Analysis was performed using statistical package SPSS 17.0 (SPSS, USA).

RESULTS

A total 300 patients with extraintestinal *E. coli* infection were included in this study. These included 159 (53%) cases of UTI, 77 (25.6%) with bacteremia, 40 (13.3%) with wound infection, 19 (6.3%) with pneumonia, 3 (1%) intravascular device infection and 2 (0.6%) with meningitis (Table 1). One hundred forty three patients were from medical unit, 44 from surgical, 43 from urology, 20 from oncology, 20 from gastroenterology, 13 from OBG, 12 from orthopedics and 5 from pediatrics units.

Demographic data of patients: Of the 300 patients, 163 (54%) were males and 137 (46%) were females with the age group of <1= 4 (1.3%), 1-18= 8 (2.6%), 18-44=71 (23.6%), 45-59=87 (29%) and >60=130 (43%). Majority were Community acquired infections, 267 (89%) and 33 (11%) were hospital acquired infections (Table 1).

Table 1. Demographic detailes of the patients infected with ESBL, AmpC and Carbapenemase producing extra-intestinal *E. coli*.

Type of ExPEC strains	ESBL producers, n=212 (%)	AmpC producers, n=95 (%)	Carbapenemase producers, n=29 (%)
Type of Infection			
UTI	109 (51.5)	48 (50.5)	19 (65.5)
Bacteremia	56 (26.5)	22 (23)	4 (14)
Wound Infection	29 (14)	15 (16)	2 (7)
Pneumonia	14 (6)	7 (7)	2 (7)
IVD Infection	2 (1)	1 (1)	0
Meningitis	2 (1)	2 (2)	2 (7)
Age Group (Years)			
< 1	3 (1.5)	1 (1)	1 (3.5)
1 - 18	6 (3)	4 (4)	0
19 - 44	48 (23)	26 (27)	8 (27.5)
45 - 59	63 (30)	31 (33)	12 (41)
> 60	92 (43)	33 (35)	8 (27.5)
Gender			
Male	119 (56)	52 (55)	19 (65.5)
Female	93 (44)	43 (45)	10 (34.5)

UTI=Urinary tract infection, IVD=Intravascular devices

ESBL producers

Of the 300 *E. coli* isolates, 212 were confirmed ESBL producers by double disk diffusion assay, indicating a prevalence of 70.6% (212/300). Of these, 97 (46%) strains of ESBL producers were from medical wards (Table 2).The clinical sites of isolation are summarized in table 1. For 185 patients the ESBL producing strains were isolated in the first 24 h after admission. The remaining patients presented with infection from 5 days up to 3 months after admission, suggesting that these were hospital acquired infections. The analysis of drug resistance pattern showed that all ESBL producers were more frequent co-resistance to other non-beta lactam classes of antibiotics (Table 2).

Table 2. Prevalence of ESBL,AmpC & Carbapenemase producing E.coli causing extra-intestinal infection.

Type of ExPEC strains	ESBL producers, n=212 (%)	AmpC producers. n=95 (%)	Carbapenemase producers, n=29 (%)
Medicine	97 (46)	42 (44)	12 (46.5)
Surgery	30 (14)	14 (15)	5 (17)
Urology	31 (15)	12 (13)	7 (24)
Gastroenterology	17 (8)	7 (7)	0
Oncology	17 (8)	7 (7)	2 (7)
OBG	10 (4)	9 (9)	1 (3.5)
Orthopedics	6 (3)	3 (3)	1 (3.5)
Pediatric	4 (2)	1 (1)	1 (3.5)
Community acquired	185 (87)	75 (79)	22 (22)
Hospital acquired	27 (13)	20 (21)	7 (24)
Resistance to non-β lactams			
Fluoroquinolones			
Ciprofloxacin	167 (79)	73 (77)	28 (96)
Norfloxacin	88 (41.5)	41 (43)	17 (59)
Sulfonamides			
Co-trimoxazole	88 (41.5)	43 (45)	16 (55)
Aminoglycosides			
Amikacin	41 (19)	24 (25)	16 (55)
Gentamicin	122 (57.5)	55 (58)	19 (65)

Note: Several strains of ExPEC produce multiple enzymes, data of which is not shown above.

AmpC producers: AmpC β lactamase production was observed in 95 (32%) isolates by the AmpC disk test. Sixteen (17%) isolates were pure AmpC producers whereas 79(83%) isolates were ESBL co-producers. Isolation wards, clinical sites, age group and drug resistance pattern to other non β lactam classes of antibiotics by the AmpC producing isolates are summarized in table 1. There is a significant difference ($p<0.05$) between ESBL and AmpC producers.

Carbapenemase producers

In this study, 29 (9.5%) isolates of *E. coli* were carbapenemase producers by disk diffusion test and modified Hodge test (Table 1). The isolates were further evaluated phenotypically for presence of metallo β- lactamase (MBL), using the metal chelating agent EDTA. Fifteen (5%) isolates were MBL positive by both combined disk tests and MBL E-test (Figure 1).

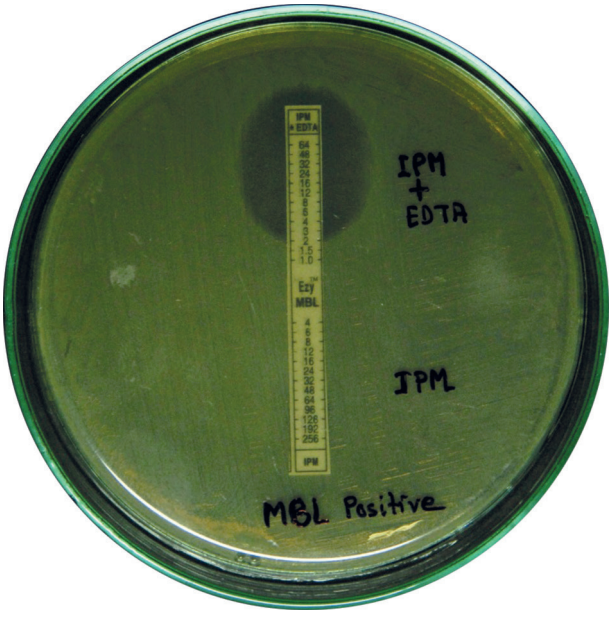

Figure 1. Detection and determination of minimum inhibitory concentration (MIC) to Imipenem by ExPEC strains.

Imipenem resistance

Of the 300 strains of ExPEC, 18 were found to be resistant to Imipenem (MIC of > 16 µg/ml) as detected by the E-test (Table 3). Eighty strains, which had MIC of 8 - 16 µg/ml were found to demonstrate intermediate resistance to imipenem. It was observed that the hospital strains of *E. coli* showed significantly higher resistance ($p<0.05$) to imipenem than community acquired strains (Table 3).

Table 3. Susceptibility patterns of ExPEC to Imipenem:

		Type of Infection						
Source	Susceptibility	UTI	Wound	Bacteremia	Pneumonia	IVD infection	Meningitis	Total (%)
Community acquired	Sensitive	57	10	37	6	0	0	110
	Intermediate	68	22	34	11	1	2	138
	Resistant	13*	0	4	0	0	0	17
	Total no. of community acquired strains	138	32	75	17	1	2	265
Hospital acquired	Sensitive	3	3	0	0	0	0	6
	Intermediate	12	4	1	2	2	2	23
	Resistant	5*	1	0	0	0	0	6
	Total no. of hospital acquired strains	20	8	1	2	2	2	35
Total no. of strains		158	40	76	19	3	4	300

Interpretation of MIC values, <8µg/ml = Sensitive; 8 - 16µg/ml = Intermediate resistance; >16µg/ml = Resistant; *No. of resistant strains of ExPEC from UTI were significantly higher ($P< 0.05$) in hospital acquired infections when compared to community acquired strains.

Treatment & Outcome

The most widely prescribed antibiotics were β lactam + β lactamase inhibitors, cephalosporins, and carbapenems. Of our study population, maximum number of patients (67%) recovered with appropriate antibiotic treatment. Relapses and re-infections were seen in 18% patients and in 11% of patients the primary cause of death was ExPEC infection. Outcome of ExPEC infection with ESBL, AmpC and carbapenemase producers are summarized in Figure 2.

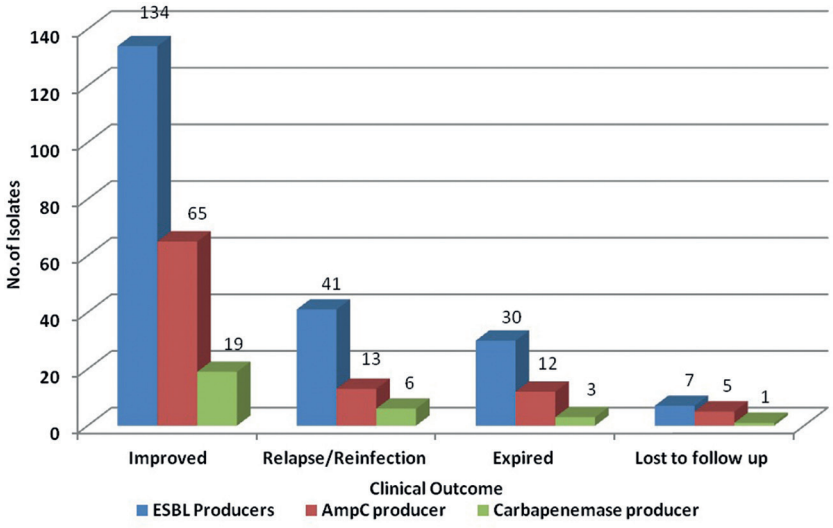

Figure 2. Outcome of infection with ExPEC producing ESBL, AmpC and Carbapenemases (Several isolates produced multiple enzymes, data not shown)

DISCUSSION

Escherichia coli is emerging as an important cause of extraintestinal infections in our hospitals. The growing increase in the rate of antibiotic resistance of these isolates is a major cause of concern. β-lactams have been the mainstay of treatment for serious infections, the most active of these being

carbapenems, which are advocated for use in treatment of infections caused by ESBL producing *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae*.[9] Pathogens that produce ESBL or AmpC β lactamases along with carbapenemases are particularly challenging for clinicians and are a major threat worldwide.

Results of our study have shown that extraintestinal infection with ESBL producing *E. coli* of hospitalized patients was 71% in our setting. Other studies from India have reported 50-70% prevalence of ESBL producing among *E. coli*.[5,10] We found most of our urine isolates were ESBL producers which was common in all age groups. ESBL producing *E. coli* were isolated from infants also. However, ESBL isolates were more common in elderly patients and from Medical units. Several studies have also reported similar results.[11,12]

Previous studies have shown that ESBL producing organisms were frequently resistant to non β-lactam antibiotics such as fluoroquinolones, cotrimoxazole and aminoglycosides.[5,13] In our study we found a high degree of resistance to multiple classes of antibiotics among ESBL producing isolates. Only carbapenem group of antibiotics were the most active (9.5% were resistant) among all antimicrobials tested.

In our study population, we found that 31.6% of isolates were AmpC producers. Other studies from India have reported a 30-50% prevalence rate of AmpC production among *E. coli*.[14,15] Seventeen percent of these isolates were pure AmpC producers. Several studies have reported about 8-15% of the isolates were pure AmpC producers.[14,15] A study from Canada has shown that the highest number of AmpC producing isolates were from urine samples, from elderly patients (>60 years) and from medical care units which was similar to our findings.[16]

Analysis of antibiograms for AmpC producing isolates revealed that 95% of strains were resistant to amoxicillin-clavulanic acid. In contrast, 43% of strains were resistant to piperacillin/tazobactam. For extended spectrum cephalosporins, 68% of strains were resistant to ceftazidime and 92.5% were resistant to cefotaxime. Several studies have shown that cephalosporin susceptibility screening of *E. coli* isolates with the initial purpose of ESBL identification resulted in selection for AmpC producing strains.[17,18] On the basis of our results, we cannot recommend extended spectrum cephalosporins as screening parameters for AmpC, which is similar to other findings.[19]

In our study, 7.5% of the ExPEC isolates were carbapenemase producers. Several studies from India have shown a prevalence rate of 8-10% of *enterobacteriaceae* isolates being carbapenemase producers.[20,21] However although only 5% of our ExPEC isolates were positive for MBL activity, it is alarming as such infections may result in mortality or chronic persistence leading to repeated hospitalization.

The problem with MBL producing isolates is their unrivalled broad-spectrum resistance profile. These MBL positive strains are usually resistant to β-lactams, aminoglycosides and fluoroquinolones. However, they usually remain susceptible to polymyxins. No extended survey with a series of human infections with MBL positive isolates has been performed to determine optimal treatment. The only alternative may be the therapeutic administration of polymyxins, which has recently been shown to be efficient for treating multidrug-resistant gram negative bacilli.[22,23] In any case, these molecules should not be used in mono-therapy and rapid determination of MICs of aminoglycosides may help to choose an aminoglycoside molecule that may have some activity. Clearly, in the absence of novel agents in the near future, the spread of MBL producers may lead to therapeutic dead ends.

In our study we found that β lactam + β lactamase inhibitor was considered the most reliable class of antibiotics for treatment of infections caused by ESBL producing ExPEC while for non ESBL producing ExPEC cephalosporins were the most prescribed antibiotics. However, for treatment of MBL producing ExPEC multiple groups of antibiotics was used.

We also found some of our patients improved with treatment of antibiotics, which were found to be resistant by the modified Kirby-Bauer disk diffusion method, and based on this finding, we recommend that for MDR isolates, MIC should be performed for higher antibiotics to reduce cost of treatment and to prevent morbidity.

Outcome of our study indicates that 67% of patients improved with proper antibiotic treatment whereas 18% patients developed relapse/re-infection and 11% of patients expired due to infection caused by multi-drug resistant *E. coli*. Mortality was significantly higher for patients with blood stream infection, which was comparable to previous studies.[24,25] However, it is difficult to demonstrate attributable mortality solely to infection without proper study design and/or autopsy to provide evidence as some patients had other underlying conditions.

In conclusion, microbiology laboratories must be able to detect resistant pathogens in a timely manner, especially those that are falsely susceptible in vitro to drugs that may be considered for therapy of infected patients. Microbiological excellence is needed more than ever, and ESBLs, AmpC β lactamases and carbapenemases production should be detect accurately. In addition, there should be good communication between the microbiologist and the health care worker to make better patient outcomes, facilitating effective infection control, reducing spread of resistant pathogens and helping hospitals to meet accreditation standards. This will help in the fight against multidrug resistance ExPEC and if corrective measures are not taken, in the absence of novel agents in the near future, the spread of MDR isolates may lead to therapeutic dead ends.

Acknowledgement: The abstract was presented at the 2nd Annual Conference of the Clinical Infectious Disease Society at Chennai India from 24th to 26th August 2012.

Disclosure: We would like to thanks Manipal University for the financial support

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