

# Detection of Carbapenem Resistance in Extended Spectrum Beta Lactamase Producing Eshcherichia Coli Isolates in a Tertiary Care Hospital

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## ABSTRACT

**Background:** Carbapenems are  $\beta$ -lactam antibiotics, presently considered as the most potent agents for the treatment of multi drug resistant gram negative bacterial infection. Their clinical usage is under threat due to growing incidence of resistance due to production of Carbapenemases which belong to Class B  $\beta$ -lactamases. Knowledge of resistance patterns for Carbapenems will help to guide appropriate and judicious antibiotic use. Hence this study was conducted to detect the resistance to Carbapenems in ESBL producing isolates of *E. coli*, as the growing incidence of Carbapenem resistance is a major concern to public health.

**Objectives:** 1. Screening and phenotypic confirmation of ESBL production in *E. coli* isolates. 2. Screening for Carbapenem resistance in ESBL producing *E. coli* isolates.

**Materials and Methods:** Clinical samples consisting of all exudates, sterile body fluids and urine samples were included in our study. Samples were processed and *E. coli* identified as per routine laboratory protocol. ESBL screening and phenotypic confirmation, followed by screening for Carbapenem resistance was done as recommended by CLSI guidelines 2014.

**Results:** We isolated 100 ESBL producing *E. coli* from the samples which were further screened for Carbapenem resistance. Among 100 ESBL producing *E. coli* isolates, 4(4%) showed Carbapenem resistance, out of which 2 were isolated from urine, 1 each from pus and stool. Co-resistance was observed to other antibiotics including other Cephalosporins(91%), Ampicillin(100%), Piperacillin(95%) and Fluoroquinolones(70%).

**Conclusion:** Our study highlights an increase in resistance pattern in ESBL producing *E. coli* isolates towards Carbapenems, which is an alarming situation to microbiologists and clinical physicians of our country. Hence, there is a need to emphasize on rational use of antimicrobials and prevent their misuse which is harmful to both patient and community.

**Keywords:** Carbapenemase,  $\beta$ -lactam, ESBL, *E. coli*, Carbapenem resistance

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## INTRODUCTION

The emergence of antibiotic resistance is a global public health problem. Gram negative bacterial resistance is of particular importance as there is a dearth of novel antibiotics directed against this organisms.<sup>1</sup>

Penicillins and Cephalosporins remained the first line of defense against microbes for over 20 years until resistance due to Beta lactamase produced by Gram negative bacilli became a serious problem.<sup>2</sup> Beta lactamase production is perhaps the single most important mechanism of resistance to Penicillins and Cephalosporins. *E. coli* possess a naturally occurring chromosomally mediated Beta lactamase or plasmid mediated Beta lactamase.<sup>3</sup>

ESBLs are plasmid mediated enzymes which are capable of hydrolysing and inactivating a

wide variety of Beta lactams including 3<sup>rd</sup> generation Cephalosporins, Penicillins and Aztreonam.<sup>4</sup> Most of these plasmids not only contain DNA encoding ESBL enzymes, but also carry genes which confer resistance to several non Beta lactam antibiotics. The most frequent coresistance found in ESBL producing organisms are Aminoglycosides, Fluoroquinolones, Tetracyclines, Chloramphenicol and Sulfamethoxazole-Trimethoprim.<sup>4</sup> This has led to a parallel increase in the usage of Beta lactamase inhibitor/ Beta lactam combinations, Monobactams and Carbapenems.<sup>5</sup>

Carbapenems are beta lactam antibiotics, presently considered as the most potent agents for the treatment of multi drug resistant Gram negative infection due to stability of these agents against majority of beta lactamases and their high rate of permeation through bacterial outer membrane.<sup>5</sup> The clinical utility of Carbapenems is under threat with the growing incidence of pan resistant isolates.<sup>1</sup>

Resistance in bacteria to Carbapenems is due to production of Carbapenem hydrolyzing enzymes called Carbapenemases. These belong to Class B of beta lactamases. This is of great concern, as presently to combat infections by multi drug resistant bacteria, Carbapenems are considered the

last resort, especially in intensive care units and high risk wards.<sup>5</sup>

Shiju et al, had conducted a similar study and have shown an increase in the incidence of ESBL producing *E. coli* and *K. pneumoniae* strains in Mangalore. However they noticed 100% sensitivity to Carbapenem (imipenem).<sup>4</sup>

Gupta et al, conducted a study in AIIMS, New Delhi, where they observed 3.5% resistance to Meropenem among the ESBL producing *Escherichia coli* isolates.<sup>5</sup>

The growing incidence of Carbapenem resistance and diversity of carbapenemase producing strains is a major concern to public health. Hence this study was conducted with an objective to screen and confirm phenotypically the production of ESBL in *E. coli* isolates and to screen for resistance to Carbapenems in ESBL producing isolates of *E. coli*.

## MATERIALS AND METHODS

1. **Source of Data:** The present study was conducted in Microbiology laboratory of McGann Hospital, attached to Shimoga Institute of Medical Sciences, Shimoga for a period of 6 months from June 2014 to December 2014. All exudates, sterile body fluids and urine samples were included in our study. Stool samples of infants were also included.

2. **Method of Collection of Data:** All samples like exudates, sterile body fluids and urine received in our Microbiology laboratory were processed.

Samples were inoculated on to Blood agar and MacConkey agar plates and incubated aerobically overnight at 37°C. On the basis of colony morphology, Gram staining, motility and biochemical reactions, the organisms were identified as *E. coli*.<sup>9</sup>

Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI) guidelines 2014. (M100-S24) Commercially available antibiotic disks (Himedia Labs, India) were used for antimicrobial susceptibility testing.

## ESBL DETECTION METHODS

*E. coli* were first screened for ESBL production by phenotypic method and then phenotypic confirmatory test was done as per CLSI guidelines 2014 (M100-S24)

1. **Phenotypic screening for ESBL resistance to beta lactams:** *E. coli* isolates were screened for resistance to 3 cephalosporins: Cefotaxime (30µg), Cefotaxime (30µg) and Ceftriaxone (30µg) by Kirby Bauer disk diffusion test. CLSI has recommended the use of any of these antibiotic disks for screening *E. coli* isolates that displayed resistance to one or more of these

antibacterials were considered positive for screening test.

2. **Phenotypic confirmatory detection of ESBL production:** Method used: 3<sup>rd</sup> generation Cephalosporin/Clavulanate combination disk test (30µg/10µg)

Presence of ESBL among *E. coli* isolates positive on screening test was further confirmed by using both Ceftazidime (30µg)/ Ceftazidime-Clavulanic acid (30µg/10µg) AND Cefotaxime (30µg)/ Cefotaxime- Clavulanic acid (30µg/10µg) disks according to phenotypic confirmatory test. An increase in zone diameter by ≥ 5mm around the disk with Cephalosporin and Clavulanic acid versus the zone around disks with Cephalosporin alone was interpreted as POSITIVE as per CLSI guidelines 2014.



3. **Screening test for suspected carbapenemase production:** ESBL producing *E. coli* isolates were screened for resistance to Carbapenem (Ertapenem 10µg/ Meropenem 10 µg) by Kirby Bauer disk diffusion test on Mueller Hinton agar media. *E. coli* isolates that displayed resistance to Ertapenem 10µg were considered positive for Carbapenem resistance in screening test. Quality controls were also used.



## RESULTS

100 ESBL producing *E. coli* isolates were included in this study without applying any selection criteria for the patients. Distribution of these isolates on the basis of the source is documented in table 1.

**Table 1: Distribution of ESBL producing *E. coli* isolates on the basis of source**

Source	Number	Percentage
Urine	41	41%
Pus	34	34%
Stool	17	17%
Blood	8	8%
Sputum	0	0%
Other fluids	0	0%

ESBL producing *E. coli* was isolated in the highest number from urine samples, followed by pus, stool and blood samples.

Among the 100 ESBL producing *E. coli* isolates, only 4 isolates showed resistance to Ertapenem (10 µg) when tested using Kirby Bauer disk diffusion test. These isolates were reported as positive for screening for Carbapenem resistance. Out of the 4 isolates, 2 were recovered from urine, 1 each was recovered from pus and stool samples. All the isolates from blood were Carbapenem sensitive. This is documented in Table 2.

**Table 2: Distribution of Carbapenem resistance in ESBL isolates of *E. coli***

SOURCE	No of Carbapenem resistant isolates (out of 100)
Urine	2
Pus	1
Stool	1
Blood	0

The ESBL producing *E. coli* isolates also showed co-resistance to other antibiotics. The resistance pattern of these isolates to other antibiotics is documented in Table.3.

**Table 3: Antibiotic resistance pattern of ESBL positive isolates of *E. coli***

Antimicrobials	Percentage of resistance
Ampicillin	100%
Cefotaxime	100%
Ceftazidime	100%
Ceftriaxone	91%
Piperacillin	95%
Fluoroquinolones	70%
Amikacin	13%
Ertapenem	4%
Imipenem	3%

## DISCUSSION

Beta lactamases are a large family of enzymes representing major mechanisms of

resistance of bacteria against beta lactam antibiotics. ESBL production by *E. coli* has become a major problem in clinical practice in last few years due to extensive use of beta lactams. The chromosomally mediated beta lactamases are inducible or constitutive non-transferrable. The second type of beta lactamases are the plasmid mediated ESBLs which are constitutively expressed and transferrable. Co-transfer of resistance against aminoglycosides, quinolones, tetracyclines and chloramphenicol is common on ESBL plasmids.<sup>8</sup>

Until recently, Carbapenems were the choice for the therapeutic management of multi drug resistant gram negative bacterial infections. Currently, the spread of Carbapenem resistant bacteria has caused grave concern due to limited choice in antibiotics for treating infections caused by them.<sup>7</sup>

Carbapenemases, both Class A (KPC) and Class B (MBL) have shown a worldwide dissemination and probably are the major contributors of Carbapenem resistance.<sup>1</sup> Most of the MBL (metallobeta lactamases) confer resistance to not only Carbapenems, but also to other beta lactamase inhibitors such as Clavulanic acid, Sulbactam and Tazobactam.<sup>5</sup> These bacteria have the potential to spread rapidly within the hospital environment and also across the continent.<sup>5</sup>

In our study, we observed 4% resistance to Carbapenems in ESBL producing *E. coli* isolates, i.e, 4 out of 100 isolates showed resistance to Ertapenem (10µg) by Kirby Bauer disk diffusion test. Gupta et al, in their study have reported 2.1% resistance to Carbapenems in ESBL producing *E. coli*. Datta et al, have also shown similar results in their study with slight variation.

We observed that the majority of Carbapenem resistant isolates were detected in urine samples, followed by pus and stool samples. Nagaraj et al, in their study have also reported most of the isolates from urine, followed by wound discharge.

The established fact that ESBL producer's shows cross resistance to other antibiotics has also been noted in our study. We observed in our study, that all the ESBL isolates were resistant to Ampicillin and majority of them were resistant to Piperacillin and Cephalosporins such as Cefipime, Cefuroxime and Ceftriaxone. However, there resistance to fluoroquinolones observed was 70%. We also noted lesser resistance to Amikacin (13%) compared to other drugs. Shiju et al, have shown similar cross resistance in their study with slight variation.

Limited literature is available regarding the prevalence of resistance to Carbapenems in various clinical isolates from our country.<sup>5</sup> Hence, screening for Carbapenemase activity by the laboratory is the first step towards early detection and continued surveillance of these pan resistant isolates.<sup>1</sup>

## CONCLUSION

To conclude, our study highlights an increase in resistance pattern in ESBL producing *E. coli* isolates towards Carbapenems, which are one of the last resort drugs for treatment of ESBL producing Gram negative bacteria. It is of grave concern that most of these isolates also exhibit co-resistance to other most commonly used antimicrobials. This is an alarming situation to microbiologists and clinical physicians of our country. Hence, there is a need to emphasize on rational use of antimicrobials after proper antimicrobial susceptibility testing and prevent misuse of antimicrobials which is harmful to both patient as well as the community.

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