



## Original Research Article

# Mechanisms of carbapenem resistance among clinical isolates of *Escherichia coli* in a tertiary care hospital

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## ARTICLE INFO

## Article history:

Received 02-01-2020

Accepted 20-02-2020

Available online 08-04-2020

## Keywords:

*Escherichia coli*

Carbapenem resistance

Super bug

Carba NP

MBL

Carbapenem resistance mechanisms

## ABSTRACT

**Introduction:** The emergence and global spread of carbapenem-resistant *E. coli* is of great concern to the health care facilities due to high rates of morbidity and mortality associated with them. It is necessary to know its epidemiology and the resistant pattern in a geographical area to formulate an antibiotic stewardship policy.

**Objective:** To study the occurrence and mechanisms of carbapenem resistance in *Escherichia coli*.

**Materials and Methods:** A total of 1172 clinical strains of *E. coli* obtained from various clinical specimens were screened for carbapenem resistance during the study period. Strains showing reduced susceptibility to imipenem &/or ertapenem &/or meropenem were included in the study. The resistance mechanisms were identified using various phenotypic methods.

**Results:** Total of 53/1172 were found to be carbapenem resistant *E. coli* (CRE). The most common sample in which CRE were isolated was urine (n=26, 49.1%). A total of 50 isolates were confirmed as Metallo-beta-lactamase (MBL) producers using Ezy MIC<sup>TM</sup> strip. One of the three non carbapenemase producing isolate was positive for ESBL with porin loss and the other two isolates were positive for AmpC with porin loss.

**Conclusion:** MBL production being the most common mechanism of carbapenem resistance, the study indicates the importance of regular monitoring of drug resistance in the hospital for an urgent action to be taken for antibiotics stewardship in the institute.

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## 1. Introduction

Bacteria are remarkably adaptable organisms that possess an almost unlimited capability to survive under adverse conditions.<sup>1</sup> One of the most effective survival mechanisms among pathogenic bacteria is development of antimicrobial resistance. Resistance to carbapenem among Enterobacteriaceae in general can be acquired through various mechanisms; most concerning are carbapenemases, enzymes produced by the organisms against carbapenems directly. As of 2015, *Klebsiella pneumoniae* carbapenemase (KPC) is the most widespread carbapenemase in the western countries while New Delhi Metallo-beta-lactamase-

1 (NDM-1) in India.<sup>2</sup> One reason for this rapid dissemination is that carbapenemase enzymes are typically located on plasmids that facilitate transmission within and between bacterial species.<sup>2</sup>

The emergence and global spread of carbapenem-resistant bacteria is of great concern to the health care facilities. These bacteria are often resistant to all other  $\beta$ -lactam antibiotics limiting the available treatment options. The carbapenem resistant Enterobacteriaceae (CRE) are associated with high rates of morbidity and mortality particularly amongst critically ill patients.<sup>3</sup>

Within the Enterobacteriaceae family, carbapenemases have been found most commonly in *Escherichia coli* and *Klebsiella pneumoniae*.<sup>4</sup> It is necessary to know the prevalence and the resistant pattern of bacterial strains in

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a geographical area so as to formulate a policy of empirical therapy in high risk units where infection due to resistant organisms is higher, and rapid detection of CRE becomes the need of the hour. Hence the study was undertaken to know the mechanisms of carbapenem resistance among clinically isolated *Escherichia coli*.

## 2. Materials and Methods

### 2.1. Strain collection and source of data

Clinical samples (urine, blood, exudates, sputum and other body fluids like pleural fluid, peritoneal fluid and pericardial fluid) that were received in the laboratory of ESIC MC & PGIMSR, Rajajinagar, Bangalore from January 2017 to June 2018 were cultured on 5% sheep blood agar and MacConkey's agar. Any growth seen was identified to species level using standard laboratory techniques.<sup>5</sup> One thousand single patient isolates of *E. coli* were studied. All other species of family Enterobacteriaceae, non-fermenting gram negative bacteria and gram-positive bacteria were excluded from the study. Antibiotic susceptibility testing was performed by Kirby Bauer disk diffusion method and interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines. Those strains which showed reduced susceptibility to imipenem &/or ertapenem &/or meropenem were studied further.<sup>6</sup>

### 2.2. Minimum Inhibitory Concentration (MIC) for Imipenem along with metallo beta lactamase (MBL) detection

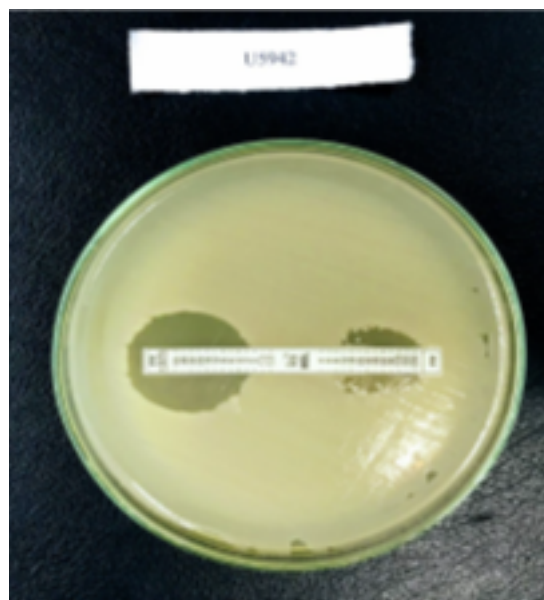
The imipenem (IPM) with and without Ethylenediaminetetraacetic acid (EDTA) Ezy MIC<sup>TM</sup> strips were obtained from Hi Media, Mumbai. The MIC detection was done according to manufacturer's instructions. The Ezy MIC<sup>TM</sup> strip was placed on the surface of the Muller Hinton agar (MHA) plate that was inoculated with the test strain as a lawn culture. Within 60 secs the strip was adsorbed and firmly adhered to the agar surface. The plates were further incubated at 35°C until sufficient growth was seen. The MIC value was read where the ellipse intersected the scale on the strip. *E. coli* ATCC 25922 was used as control organism.

### 2.3. Interpretation for MBL detection

MBL positive strain – when the ratio of the value obtained for IPM: the value of IPM+EDTA is more than 8 or if zone is observed on the side coated with IPM+EDTA and no zone is observed on the IPM side (Figure 1)

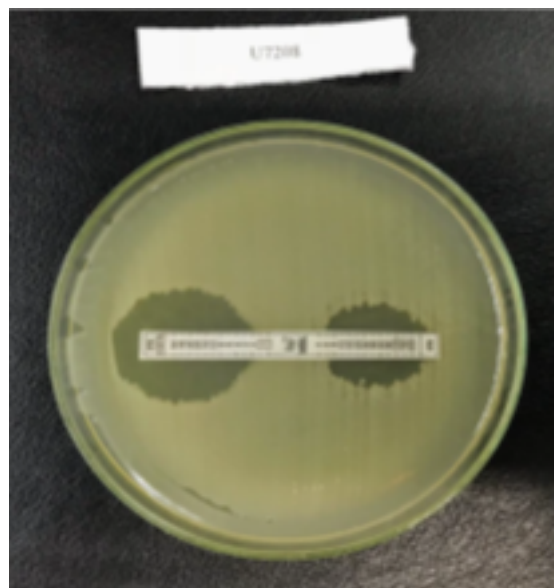
MBL negative strain – when the ratio of the value obtained for IPM : the value of IPM+EDTA is less than or equal to 8 (Figure 2)

MBL non-conclusive – when no zone of inhibition is obtained on either side (resistance may be due to other mechanisms) or if complete inhibition is obtained on both



**Fig. 1:** Ezy MIC<sup>TM</sup> strips – MBL positive

the sides of the strip.

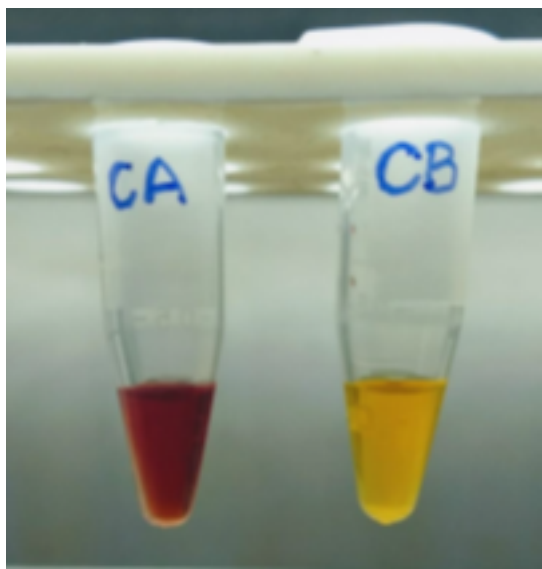


**Fig. 2:** Ezy MIC<sup>TM</sup> strips – MBL negative

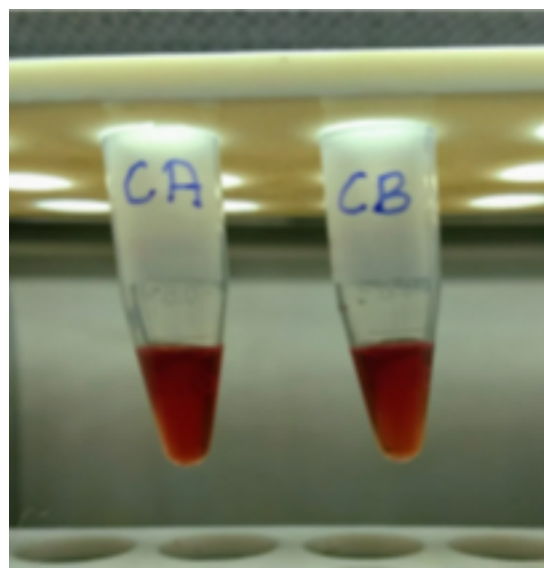
### 2.4. CarbaNP:<sup>6</sup>

CarbaNP (CNP) A solution was prepared by adding phenol red (0.05%) and ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.1 mmol/L) to Clinical Laboratory Reagent Water; pH was adjusted to 7.8 ± 0.1, and the solution was stored at 4°C in amber-coloured bottles for up to 15 days. The B solution was freshly prepared by adding 12mg/ml imipenem-cilastatin injectable form (doubling the amount to compensate the cilastatin

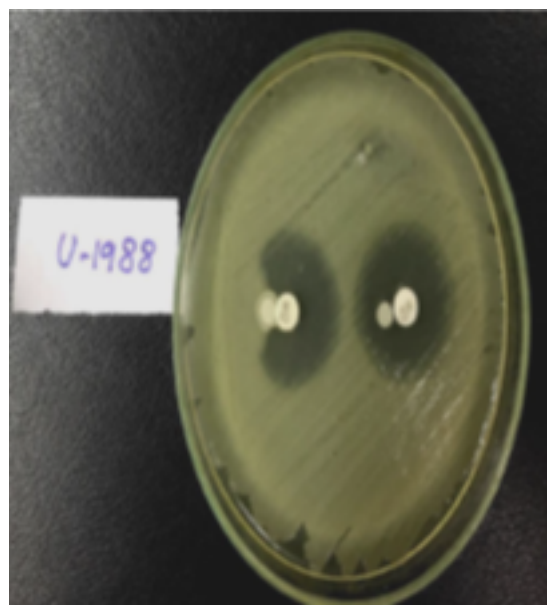
component; equivalent to 6mg/ml of imipenem standard grade powder) to A solution and stored at 4°C till use. A calibrated 10µL loop full of test strain from 18 to 24 hours culture from sheep blood agar was resuspended in a 200µL of 20 mM/LTris-HCL lysis buffer, vortexed for 1 minute and incubated at room temperature for 30 minutes. Bacterial lysate (100 µl) was added to two microcentrifuge tubes labeled “a” and “b.” Reagents A and B were added to tubes a and b, respectively, incubated at 37°C and readings were taken at 10 min, 30 min, and 120 min by three different observers. *Klebsiella pneumoniae* ATCC 1705 and *Klebsiella pneumoniae* ATCC 1706 were used as positive and negative controls respectively. The test was considered positive when tube “a” was red and tube “b” was orange/yellow (Figure 3). In a negative test, both tubes remained red (Figure 4).



**Fig. 3:** Carba NP test –positive



**Fig. 4:** Carba NP test – negative



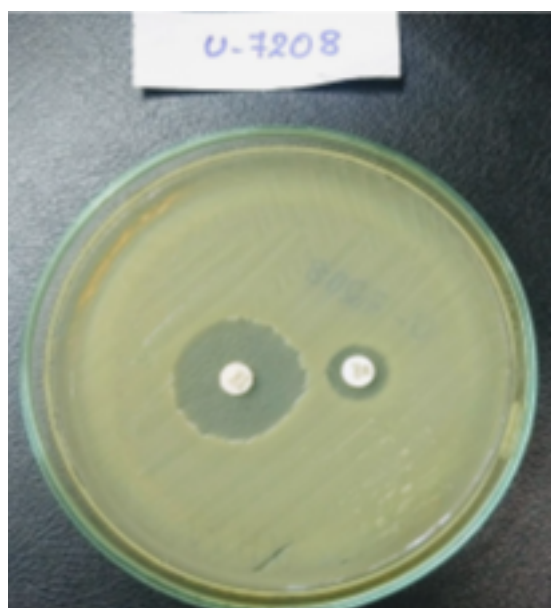
**Fig. 5:** AmpC disc test

## 2.5. Detection of AmpC beta lactamase

AmpC disk test was done according to Black *et al.* A lawn of *E. coli* ATCC 25922 was made on MHA. A 30µg cefoxitin disk was kept on the surface of the agar. A blank filter paper disk (impregnated with 10x tris-EDTA) was inoculated with few colonies of test strain and placed beside the cefoxitin disk almost touching to it and incubated overnight at 37°C. Flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disk with test strain was considered positive for the production of AmpC  $\beta$  lactamase. The undistorted zone of inhibition was considered as negative. Known AmpC positive and negative stains are used as controls.<sup>7</sup> (Figure 5)

## 2.6. Phenotypic confirmation for detection of ESBL:<sup>6</sup>

Extended spectrum beta-lactamase (ESBL) production was detected by using CLSI described phenotypic confirmatory test using cefotaxime (CTX) 30mcg and ceftazidime (CTZ) 30mcg alone and in combination with clavulanic acid (CA) 10mcgs. Standard Disk diffusion procedure was followed and the disks were placed on MHA on which a lawn culture of the test organism was done. The plates were incubated for 18-24 hours at 37°C. After overnight incubation reading was taken and zone of inhibition was read. A  $\geq 5$ mm increase in a zone diameter for the cephalosporin with clavulanate was



**Fig. 6:** Phenotypic detection of ESBL

considered as confirmation of ESBL production. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 was used as positive and negative controls, respectively. (Figure 6)

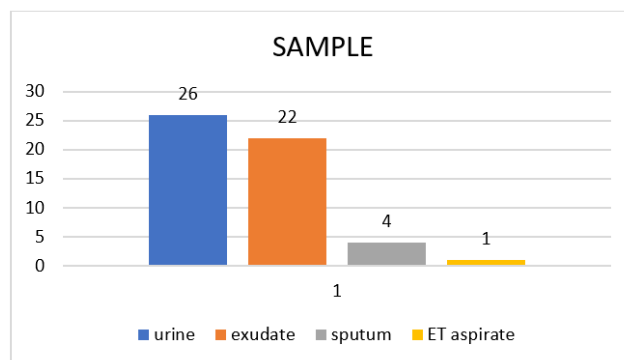
### 2.7. Efflux pump over expression using Carbonyl cyanide 3-chlorophenylhydrazone (CCCP)

CCCP was incorporated in MHA at concentrations of 12.5  $\mu$ M and meropenem susceptibility testing by disc diffusion method was performed in parallel in agar plates with and without CCCP. The result was considered positive if the inhibition zone of meropenem was wider in the agar plate with CCCP than the one in the plate without pump inhibitor.<sup>8</sup>

## 3. Results and Discussion

A total of 1172 non-duplicate isolates of *E. coli* from various clinical samples were screened during the study period. Out of these 1172 isolates, 53 isolates were found to be carbapenem resistant (CRE). The most common sample in which CRE were isolated was urine (n=26, 49.1%), followed by exudates (n=22, 41.5%) (Figure 7)

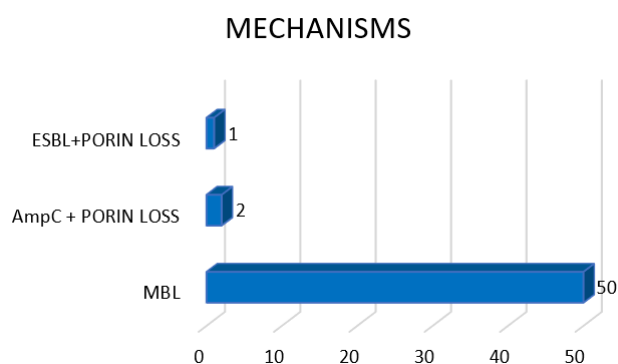
The MIC for imipenem ranged between 6  $\mu$ g/ml to 256  $\mu$ g/ml. The median MIC value, MIC<sub>50</sub> and MIC<sub>90</sub> for the CRE isolates in the study was found to be 256  $\mu$ g/ml. On the EDTA impregnated part of the strip, the MIC values were reduced up to 1.5 to 8  $\mu$ g/ml. The ratio of imipenem and imipenem+EDTA fell in the range of 1 to 171. A total of 50 isolates showed a ratio of >8 and were confirmed as MBL producers.



**Fig. 7:** Bar diagram showing the distribution of samples

The CarbaNP test was done for all the CRE isolates. Out of the 53 isolates, 49 gave positive reaction by the carbaNP test. These 49 carbaNP positive isolates were found to be MBL positive according to E strip-based test. One isolate which was confirmed as MBL producer could not be detected by CarbaNP test. The sensitivity of CarbaNP test in detecting carbapenemases was found to be 98%.

Among the 53 CRE isolates, 3 isolates were negative for carbapenemases. These were further studied for other mechanisms of carbapenem resistance. These isolates were tested for phenotypic confirmation for ESBL and AmpC. One of the three isolate was positive for ESBL and the other two isolates were positive for AmpC. All the three isolates were ceftazidime resistant and hence presumptively considered to have loss of porin channel. None of the isolates had enhanced efflux pump as mechanism of carbapenem resistance. (Figure 8)



**Fig. 8:** Various mechanisms of carbapenem resistance

Infections caused by carbapenem-resistant organisms are an emerging problem associated with high rates of morbidity and mortality, particularly amongst critically ill patients. The carbapenem-resistant organisms are usually resistant not only to  $\beta$ -lactam antibiotics but also to most other classes of antimicrobial agents. In the present study, *E. coli* was chosen as it is the most common organism isolated

from clinical specimens.

A total of 1172 non-duplicate isolates of *E. coli* were studied and 53 (4.52%) were found to be carbapenem resistant. The percentage of CRE isolated varies when compared to other studies. In a study reported by Swaminathan *et al.*, the prevalence of carbapenem-resistant Enterobacteriaceae was 5.4%.<sup>9</sup> whereas, Yi Li *et al.*, reported 18.1% of Enterobacteriaceae to be carbapenem resistant.<sup>10</sup> Hence the occurrence of carbapenem resistant organisms varies from region to region.

In this study, all the 53 CRE isolates had imipenem MIC ranging from 6 µg/ml to 256 µg/ml. The median MIC value, MIC<sub>50</sub> and MIC<sub>90</sub> for the isolates was found to be 256 µg/ml. This was in comparison with the study conducted by Fomda *et al.*, where MIC of imipenem for CRE was ≥32 µg/ml.<sup>11</sup> Out of 53 CRE isolates, 50 (94.33%) were found to be MBL producers. Higher prevalence of MBL in the present study is alarming and early intervention is required to contain the transmission to susceptible individuals. The MBL enzymes are located on transmissible plasmids. These genes are easily exchanged between bacteria. Higher occurrence of MBL in the present study indicate easy transfer of genes within or outside the Enterobacteriaceae.

The carbaNP test showed a sensitivity of 98% and specificity of 100% by picking up 49/50 carbapenemase (MBL) producing *E. coli*. It did not yield any false positive results. Whereas, in a study conducted by Rudresh *et al.*, the sensitivity and specificity of carbaNP test was found to be 77.7% and 100% respectively. Hence the carbaNP test gave consistent results with respect to its specificity, but there was a discrepancy with regards to its sensitivity.<sup>12</sup>

Several recent studies from Asian countries also demonstrated increasing incidence of MBL production in Enterobacteriaceae isolates.<sup>13–15</sup> In general, prevalence of MBL in *E. coli* isolates is increasing and the prevalence rate may vary greatly in different geographical areas and from institute to institute. In our hospital setting, MBL was more prevalent. The ESBL and AmpC with porin loss accounted for 1.9% and 3.8% of the total CRE isolates. This is in contrary to a study conducted by Kandeel A where ESBL and AmpC with porin loss accounted for 81.5%.<sup>16</sup>

#### 4. Conclusion

The most common mechanism of carbapenem resistance among *E. coli* was found to be MBL (carbapenemase) production. As the genes responsible for MBL production are present on mobile genetic elements, they can be easily transmitted horizontally between the organisms.

Most of the patients had discharging wound or urinary tract infection. They can easily transmit the infection to others either through fomites or through general toilets in the hospital. Isolation of these patients and proper treatment of underlying condition will break the chain of transmission

of CRE.

The present study also indicates the importance of regular monitoring of drug resistance in the hospital for an urgent action to be taken for antibiotics stewardship in the institute. Awareness of CRE entry into the hospital environment together with strict infection control measures will help limiting their further spread which can be difficult to control if they evolved to endemicity.

#### 5. Limitations of the Study

Our study had few limitations. The molecular mechanisms of carbapenem resistance were not detected. Only *E. coli* isolates were considered instead of entire Enterobacteriaceae. Further studies are required to know the occurrence of carbapenem resistance in other organisms. A case control study involving multiple centres and over a longer period may help to overcome the problem of small sample size.

#### 6. Source of Funding

None.

#### 7. Conflict of Interest

None.

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**Cite this article:** Ved NC, Rudresh S M , Ravi G S , Malini J , Yoganand R. **Mechanisms of carbapenem resistance among clinical isolates of *Escherichia coli* in a tertiary care hospital.** *Indian J Microbiol Res* 2020;7(1):70-75.