



## Detection of Carbapenem resistance by Carbapenem inactivation method in tertiary care centre in central Kerala

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### Abstract

**Background:** Most laboratories do not presently conduct testing for the presence of ESBL, Amp C and Carbapenemases and the justification is that these resistance genes are already well dispersed. This study reveals that if this is totally neglected, there will not be a sense of direction regarding antibiotic susceptibility and resistance patterns pertaining to the concerned scenario and consequently, the injudicious use of higher antibiotics may continue, eventually affecting patient care.

**Objective:** To determine the phenotype prevalence of Carbapenem resistant organisms in lower respiratory tract specimens at tertiary care centre in central Kerala using Carbapenem inactivation method.

**Method:** 1101 respiratory tract specimens including sputum, bronchoalveolar lavage and tracheal aspirate were received from October 2020 to March 2021. Following routine antibiotic susceptibility of all isolates to standard antibiotics by disc diffusion method according to Clinical Laboratory Standards Institute guidelines and phenotypic screening for ESBL, Amp C and Carbapenemase with the resistant isolates, Carbapenem inactivation method of testing for Carbapenemase was performed.

**Results:** Out of 301 isolates, 49 isolates were resistant to Carbapenem on routine antibiotic susceptibility testing and phenotypic screening. Among 49 isolates, 36 isolates were identified as *Klebsiella pneumonia*, 7 as *Escherichia coli*, 4 as *Acinetobacter* species and 2 as *Pseudomonas aeruginosa*. The Enterobacteriaceae namely *Klebsiella pneumonia* and *E coli* isolates were subjected to Carbapenem inactivation method for Carbapenemase testing and among these, 4 isolates (13.88%) were positive for the presence of Metallo-Beta-lactamase and only one isolate possessed Serine carbapenemase.

**Conclusion:** In the present scenario in which antibiotic resistance testing is carried out only in very limited centres and genotype testing reveals very few number of positive isolates, it will be prudent and wiser to conduct Carbapenem inactivation method of Carbapenemase testing as this will provide an awareness regarding the actual Carbapenem resistance patterns prevalent in the concerned centre.

**Keywords:** Carbapenem-resistant Enterobacteriaceae (CRE), Carbapenemase-producing Enterobacteriaceae (CPE), *Klebsiella pneumonia*, *Escherichia coli*, Metallo-beta-lactamase, Serine carbapenemase.

## Introduction

It is said that direction is more important speed, nevertheless when applied to antibiotic susceptibility, both direction and speed are equally important. The term 'pathos' means suffering and whether the pathogen arises from the patient's indigenous flora or hospital flora, it contributes to morbidity and mortality in many cases. This study discusses the resistance patterns of Gram negative bacilli isolates from respiratory specimens to Carbapenems, in a tertiary care centre in central Kerala.

These days, most of the antibiotic resistance genes are already well dispersed such as those of ESBL, Amp C and Carbapenemases, hence, many laboratories in Kerala, generally, do not presently test for their resistance mechanisms. But if this preliminary screening and phenotypic confirmatory testing of antibiotic resistance mechanisms is totally not done, there will not be a sense of direction and everything concerned, particularly the aspects of patient care, will be left in the dark.

The plasmid mediated horizontal transmission of Carbapenemases is mainly responsible for the prevalence of Carbapenem resistant Enterobacteriaceae (CRE). According to the Ambler classification method in 1980 in Mandell's Textbook of Infectious Diseases, CRE is divided into classes A, B and D<sup>1</sup>. Among these, A and D are serine  $\beta$ -lactamases while B are metallo  $\beta$ -lactamases. According to a review conducted by A M Queenan et al, Class A includes the serine carbapenemases namely KPC, IMI and SME enzymes<sup>2</sup>. Among these, KPC spreads the maximum as it is located on plasmids and is also mostly present in Klebsiella pneumonia. Class B includes the metallo-beta-lactamases namely IMP, VIM, SIM, and NDM enzymes, located as gene cassettes within integrons. Genetic transfer between bacteria can occur when these integrons associate with plasmids or transposons. Class D includes serine carbapenemases namely OXA. It is also widely accepted that there exist other mechanisms of

Carbapenem resistance such asporin loss and proton pump efflux.

In the current scenario in most places in the world, with regard to reference, there is a large overlap between CRE and Carbapenemase producing Enterobacteriaceae (CPE). The difference is that CRE were named according to Carbapenem resistant phenotype while CPE were named according to the resistance mechanism that is Carbapenemase production. It is true that rapid detection of CPE are important in the management of serious clinical infections but how far a correct distinction between CRE & CPE is important in the same is not yet clear.

Here, this study highlights that routine antibiotic susceptibility alone is not adequate with respect to Carbapenem resistance in terms of accuracy and that atleast one phenotypic confirmatory methods of Carbapenemase detection such as Carbapenem inactivation method namely 'mcim and ecim' is essential before terming an isolate as resistant to Carbapenems.

## Materials and Methods

### Bacterial Isolates

Respiratory tract specimens which included sputum, bronchoalveolar lavage and tracheal aspirate were collected for 6 months duration. Among those that yielded a positive culture, routine antibiotic susceptibility testing was conducted and those isolates resistant to Carbapenems were made to undergo a phenotypic confirmatory testing for Carbapenemase production namely 'mcim and ecim'.

### Antibiotic Susceptibility Testing

The susceptibility of all isolates to routine first line, second line and third line antibiotics were performed by disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines<sup>3</sup>. The antibiotics tested include Ampicillin, Cephalexin, Cefotaxime, Gentamicin, Amikacin, Ciprofloxacin, Cefoperazone-Sulbactam, Piperacillin-Tazobactam and Meropenem. The control strains used were

*Klebsiella pneumoniae* 700603 and *E coli* ATCC 25922.

### Detection of Antibiotic resistance mechanisms by phenotypic methods

#### Phenotypic Screening Method for ESBL, Amp C and Carbapenemase production:

On a 150mm diameter MHA plate, the following discs were placed after a lawn culture of the test organism-Aztreonam 30µg, Ceftazidime 30µg, Ceftazidime + Clavulanate 30/10µg, Cefotaxime 30µg, Cefotaxime + Clavulanate 30/10µg, Cefoxitin 30µg, Cefotetan 30µg, Ceftriaxone 30µg, Cefipime 30µg, Ertapenem 10µg, Imipenem 10µg and Meropenem 10µg. and this plate was interpreted following overnight incubation.

#### Phenotypic Confirmatory Method for Carbapenemase production

Procedure for mcim: The organism from an overnight blood agar plate is emulsified in Trypticase soy broth, Meropenem disc is immersed, the disc is then placed on Mueller Hinton agar plate inoculated with *E coli*. An inhibition zone of 6-15mm or colonies within the 16-18mm zone is positive because this indicates hydrolysis of Meropenem by the organism while zone size of  $\geq 19$ mm is negative.

Procedure for ecim: This is interpreted alongwith mcim. The procedure is the same except that EDTA is added to Trypticase soy broth. A 5 mm zone diameter difference between ecim and mcim indicates that the organism is a Metallo-Beta-lactamase producer while  $\leq 4$ mm zone size indicates ecim as negative and that the organism is a Serine carbapenemase producer.

Control strains used for the above include *Klebsiella pneumonia* ATCC BAA-1705 Serine-beta-lactamase producer, *Klebsiella pneumonia* ATCC-BAA 1706 Carbapenemase negative and *Klebsiella pneumonia* ATCC-BAA-2146 as Metallo-beta-lactamase producer.

### Results

1101 specimens were collected from lower respiratory tract infections which included sputum, tracheal aspirate and bronchoalveolar lavage. The most common specimen was sputum accounting for 65% of specimens followed by tracheal aspirate 20% and 15 % broncho-alveolar lavage. Tracheal aspirate specimens were from Critical care unit- medical and surgical side while broncho-alveolar lavage specimens were from Pulmonology.

Among, 1101 specimens received from October 2020 to March 2021, only 301 isolates yielded a positive culture, further, among these, only 49 isolates (16.27%) were resistant to Carbapenems on routine antibiotic susceptibility testing. Among these, 36 isolates were identified as *Klebsiella pneumonia*, 7 as *Escherichia coli*, 4 as *Acinetobacter* species and 2 as *Pseudomonas aeruginosa*.

The phenotypic screening method of 12 discs showed resistant zone diameter for all isolates suggesting possible Carbapenem resistance.

The phenotypic confirmatory method chosen was Carbapenem inactivation method, 'mcim and ecim'. All except 5 isolates (13.88%) were positive for presence of Carbapenemase enzyme. Figure 1 shows that the isolate of *Klebsiella pneumoniae* 402 gives a zone diameter of 7mm for mcim and 22mm for ecim. Since the difference is greater than 5mm, this indicates the isolate to be a Metallo-Beta-lactamase producer. In all, 4 isolates were metallo-beta-lactamases and only one was serine carbapenemase producer.



**Fig 1.** Isolate 402 *Klebsiella pneumoniae*, Zone size mcim 7mm and ecim is 22mm, positive for Metallo-Beta-lactamase. Isolates 399 and 400 are negative for Carbapenemase production.

Regarding the two *Pseudomonas aeruginosa* isolates, both were negative for Carbapenemase production.

### Discussion

With due regard to Carbapenems, particularly following the advent of New Delhi metallo-beta-lactamase, many articles from the Western world tend to shift the blame of current Carbapenem resistance onto the Eastern world<sup>4</sup>. This study attempts to provide a clear picture of the reality. Many studies conducted in India do not show a very high prevalence of Carbapenemase production in Enterobacteriaceae and other organisms. This may be due to the emphasis and strict adherence to Infection Control practices everywhere. It also seems more likely that other mechanisms of Carbapenem resistance such as porin loss and proton pump efflux are actually responsible for this phenomenon.

Furthermore, even though there are newspaper reports and wide social media awareness of the menace created by these superbugs, the truth is far away. The phenotype screening method showed

resistant zones for all isolates and this best only suggests Carbapenem resistance, but this cannot exactly detect ESBL and Amp C production. Unlike in most studies, here, the percentage of Carbapenemase by screening is 16.27% and by phenotypic confirmatory method is 13.88%. In a previous study in the same centre, the phenotype prevalence was 14% of *Klebsiella pneumoniae* and genotype prevalence was 9.67%<sup>5</sup>.

Both the multidrug resistant isolates of *Pseudomonas aeruginosa* were negative for Carbapenemase production and this is consistent with most studies which suggest proton pump efflux to be the main factor responsible for Carbapenem resistance.

Therefore, this study highlights the importance of conducting phenotypic confirmatory testing for Carbapenem resistance<sup>6,7</sup>. Anyway, the number of resistant strains is minimal and it is feasible and economical to perform these tests. Furthermore it doesn't require any technical skill other than that of a microbiology laboratory technician or assistant and it is not expensive like genotyping. Compared to other studies from India, here the prevalence reported is less. One reason is probably other mechanisms of Carbapenem resistance such as overproduction of ESBL and Amp C, porin loss and proton pump efflux. Another contributing factor is increased awareness and strict compliance to infection control practices.

This is a simple practical approach to determine the true Carbapenemase producers in Enterobacteriaceae in a setting and this will definitely have a positive impact on critical care patients who usually present with other comorbidities as well<sup>5</sup>. Performing phenotypic confirmatory tests of this kind will contribute in alleviating the suffering in such patients and will help in bringing down morbidity and mortality.

### Conclusion

Even though there is wide awareness of antibiotic resistance, as a step in prevention, the first measure to be taken is the accurate reporting of the antibiotic susceptibility. This study proves that

along with routine testing, at least one phenotypic screening should be done to get a clear picture of Carbapenem resistance patterns. A phenotypic confirmatory test such as Carbapenem inactivation method can contribute and this feasible approach will go a long way in issuing accurate reports of antibiotic susceptibility and decreasing the suffering of critical care patients.

**Conflict of Interest:** Nil

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**References:**

1. Mandell GL, Bennett JE, Dolin R. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 2015, 8<sup>th</sup> edition.
2. AM Queenan, Karen Bush. Carbapenemases: the Versatile  $\beta$ -Lactamses. Clinical Microbiology Reviews; July 2007: 440-458.
3. Clinical and Laboratory Standards Institute. M100 Performance Standards for Antimicrobial Susceptibility Testing 29<sup>th</sup> edition.
4. David M Livermore. Fourteen Years in Resistance. International Journal of Antimicrobial Agents. 2012 Vol 39: 283-294.
5. Sheeba K Thomas, Leah Thomas et al. Phenotype and Genotype Prevalence of Carbapenemase genes in bacterial isolates from lower respiratory tract specimens in a tertiary care centre in central Kerala. Journal of Medical Sciences and Clinical Research. September 2019, Vol 07 Issue 09: 549-553.
6. Nordmann P, Poirel L, Dortet L. Rapid Detection of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2012 Sep;18(9):1503-7.
7. Xiaoyan Cui, Haifang Zhang, Hong Du. Carbapenemases in Enterobacteriaceae: Detection and Antimicrobial Therapy. Frontiers in Microbiology August 2019, Vol 10: Article 1823.