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Original Research Article

Prevalence of Carbapenamase Resistant Kebseilla Pneumoniae and Escherichia Coli by Modified Carbapenem Inactivation Method in a Tertiary Care Hospital of North India.

Shubhdeep Kaur[1], Harsh Yadav[2], Shailesh Kawale[3], Arun Rajawat[4]

[1]Associate Professor, Department of Microbiology, Maharishi Markendeshwar College of Medical Sciences and Research, Sadopur, Ambala (Haryana)
[2]Associate Professor, Department of Pharmacology, Government Medical College, Bharatpur (Rajasthan)
[3]Professor, Department of Community Medicine, Government Medical College, Bharatpur (Rajasthan)
[4]Assistant Professor Department of Biochemistry, Mahatma Gandhi Medical College and Hospital, Jaipur (Rajasthan)

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Corresponding author: Dr. Shubhdeep Kaur
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Abstract

Background: Carbapenems are the last resort of antibiotics used in an infection caused by multidrug-resistant Enterobacteriaceae, but current increase in the prevalence of carbapenemase producing Enterobacteriaceae pose a clinical challenge. Therefore, early detection of these drug resistant organisms is important for instituting early end effective treatment to the patient to prevent from various complications. Therefore, rapid and accurate methods to detect these organisms in any clinical microbiology laboratory, including those in resource-limited settings, are essential to prevent and contain their spread.

Methods: The present study was cross sectional descriptive study was conducted in the department of microbiology for period of six months. The samples of indoor patients were included in the study and processed by conventional microbiological methods. All the isolates of *Kebseilla pneumoniae* and *Escherichia coli* isolated from various samples, which were resistant to the carbapenems (Meropenem) were further subjected to the mCIM test to detect the carbapenemase production as per CLSI M100 2017 standards.

Results: A total 550 Gram Negative Isolates, 295 were isolates of *Kebseilla pneumoniae* and *Escherichia coli.* Out of these 295 isolates 37% and 27.4% of *Kebseilla pneumoniae* and *Escherichia coli* showed the production of Carbapenemase enzyme.

Conclusions: The prevalence of CRE has been emerged worldwide. So, formulating an antimicrobial policy with its strict implementation and regular surveillance must be establish in every institution to decrease its emergence.

Keywords: Modified Carbapenem Inactivation Method (mCIM), Kirby-Bauer Disk Diffusion Method, Carbapenem Resistant Enterobacteriaceae,

Introduction

Carbapenemase-resistant Enterobacteriaceae (CRE) cause many serious infections resulting in increasing treatment cost, prolonged hospitalization, and mortality rate. Reduced expression and/or mutations of porins and the presence of carbapenemase promote Enterobacteriaceae survival under carbapenem treatments [1]. Carbapenems (imipenem, ertapenem, meropenem, and doripenem) are considered as "antibiotics of last-resort" in the treatment of critically ill patients with a variety of bacterial infections due to their broad spectrum among β-lactam antibiotics and relative resistance to hydrolysis by most β-lactamases [2,3].

The mechanisms underlying carbapenem resistance is complex.

Resistance to carbapenem is mostly mediated by production of carbapenemase enzymes, followed by chromosomal mediated porin loss and efflux pump over expression. Many carbapenemases are carried on mobile genetic elements that facilitate horizontal transfer of resistance between the Gram-negative organisms. So, the distinction between carbapenemase producing Carbapenem resistant

organisms and non carbapenemase producing Carbapenem resistant organisms is important for the infection control and epidemiological purposes. Ambler has grouped the carbapenemases on basis of their amino acid homoogy class A, B, or D. Class A (e.g., KPC) and D (e.g., OXA-48- type) enzymes possess a serine-based hydrolytic mechanism, while class B enzymes (e.g., IMP, NDM, and VIM enzymes) are metallo-β-lactamases (MBLs) that require zinc ions for catalysis and are inhibited by metal-chelating agents such as EDTA [6,7]. Several phenotypic methods for detection of carbapenemase producing isolates have been developed and used in clinical microbiology laboratories [4]. CLSI has recommended the modified Carbapenem Inactivation method (mCIM), which is effective in detecting a variety of carbapenemase in most routine microbiology laboratories in 2017 [5]. Hence, this study was conducted to detect the carbapenemase production among two members of family Enterobacteriaceae *Kebseilla pneumoniae* and *Escherichia coli* using m CIM.

Material and methods

This 6 month cross sectional descriptive study was conducted in the department of microbiology from January 2018 to June 2018. Samples of the admitted patient both of ward and ICU were included in the study. The samples from the outdoor patients were excluded. The samples were processed by convectional microbiology techniques and identified by biochemical reactions. Samples which showed mixed growth or were contaminated were excluded. All the isolates of *Kebseilla pneumoniae* and *Escherichia coli* which were resistant to the carbapenems (Meropenem) as per CLSI M100 2017 standards were included in the study. These isolates were further subjected to the mCIM test to detect the carbapenemase production as per CLSI M100 2017 standards.

Figure 1: mCIM showing positive and negative result

Modified Carbapenem Inactivation Method (mCIM)

Kebseilla pneumonia and *Escherichia coli* isolates found resistant to carbapenem by the Kirby Bauer disc diffusion was sub-culture on blood agar plate and incubated at 35ºC for 18 to 24 hours. From the sub-cultured plate 1 µl loopful of isolated colony was taken and suspended in a 2 ml of trypticase soy broth (TSB). The mixture was vortexed, 10 µg meropenem (carbapenem) disc was added and was incubated for 4 hours at 35º C. Just prior to completion of 4 hours incubation, a 0.5 McFarland suspension of Escherichia coli ATCC 25922 was prepared and lawn cultured onto a Mueller Hinton Agar (MHA) plate. After the completion of 4 hours the meropenem disc was removed from the mixture using a 10 µl loop, taking care to remove excess liquid from the disc and then the meropenem disc was immediately placed on Escherichia coli ATCC 25922 prepared MHA plate. This plate was then incubated overnight (18 – 24 hrs.) at 35º C. Next day the size of the zone of inhibition was measured. Zone size ≥ 19 mm was taken as negative and a zone size of 6 – 15 mm or presence of pinpoint colonies within a 16 - 18 mm zone was taken as positive for carbapenemase producing Enterobacteriaceae (figure 1) as per CLSI M100 2017 27th Edition guidelines.

Results

Total of 450 samples were received in the microbiology department. The distribution of various samples received from indoor patients is as follow:- urine 156(34.6%), blood 88 (19.5%), tracheal aspirates 65 (14.4%), pus 53 (11.7%), sputum 51 (11.3%), wound swab 23 (5.1%), others 14 (3.1%). Total of 550 Gram negative bacilli isolated during the study period of which 295 was the isolates of *Kebseilla pneumoniae* (166) and *Escherichia coli* (129). Out of these 295 isolates 135 showed resistant to Meropenem as per disk diffusion method CLSI 2017 criteria. 87 (64.4%) isolates out of 135 were mCIM positive and 48 (35.5%) were mCIM negative. 37% and 27.4% of *Kebseilla pneumoniae* and *Escherichia coli* showed the production of Carbapenemase enzyme.

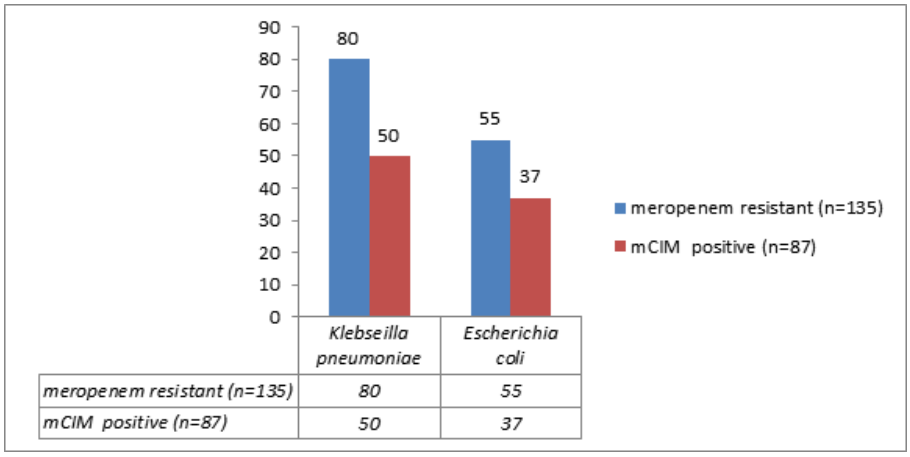

Figure 2: Distribution of Meropenem resistant isolates and mCIM positive isolates

Discussion

Enterobacteriaceae is a family of Gram negative rods and the most common isolates of this family are *Kebseilla pneumoniae* and *Escherichia coli*. The emergence of antimicrobial resistance among *Enterobacteriaceae* isolates has been increasingly reported worldwide and has become a major threat to the provision of healthcare. Carbapenems is beta-lactam antibiotics which are considered as a last line of therapy for multidrug resistant. The occurrence of carbapenem resistance among *Kebseilla pneumoniae* and *Escherichia coli* is a major health challenge which reduces the antibiotics choices that use to treat the infections which cause by these bacteria [8]. The production of carbapenemase among Gram negative bacilli varies greatly from country to country and different institutions within the country. Phenotypic assays which currently used in clinical practice to detect carbapenemase production include growth-based assays, hydrolysis methods and lateral flow immunoassay [4].

Carbapenem Inactivation method (CIM) was first described in 2015. This test is based on the promise that when 10µg meropenem disc is incubated for 2 hours in water with 10µl loop of carbapenemase producing isolates, meropenem will be hydrolysed. Initial investigations suggested that the CIM have limitations with detection of OXA type carbapenemases and MBL enzymes. But CLSI in 2017 recommonded Modified Carbapenem Inactivation method, a new phenotypic method for detection of carbapenemase production among Gram negative bacilli [4,9].

The three most common samples in our study were urine 34.6%, blood 19.6%and tracheal aspirates 14.4%, whereas in the study conducted by Giri S et al., three most common samples were urine, blood and pus each constituting 29.3%, 20%, and 11.3% respectively [10]. The carbapenem resistance in the present study was 64.4% which was much higher as compared to the study done by Jinsha K.M. et al., which was 20.3% [4]. Studies from other parts of India have reported that carbapenem resistance ranges from 9 to 22% among Gram negative bacilli [11,13]. However in the treatment guidelines document released by the Indian Council of Medical Research surveillance data a high meropenem resistance of 42%, 47% and 62% was reported among members of *Enterobacteriaceae, P. aeruginosa and A. baumannii*, respectively [14]. In our study the *Kebseilla pneumoniae* (37%) has shown the maximum carbapenem resistance as compared *Escherichia coli* (27.4%). The two most common CRE organisms reported by Giri S in his study was Klebsiella pneumonia (58%) and Escherichia coli (32 %). Our findings also corroborates with various other studies. Bo Gao et al. showed *Klebsiella pneumoniae* (44.8%) the most common, followed by *Escherichia coli* (25.8%) and *Enterobacter cloacae*. (13.8%).[15] Ravikant Porwal et al., showed two most common Carbapenem resistant *Enterobacteriaceae* in ICU setting to be *Klebsiella pneumoniae* 44% and *Escherichia coli* 26%. [16] Another study Satyajeet K Pawar et al., also showed *Klebsiella pneumoniae* (63%) and *Escherichia coli* (19%) the two most common Carbapenem resistant *Enterobacteriaceae* species isolated [17].

Kirby Bauer disk diffusion and mCIM both are phenotypic tests used to detect carbapenem resistance in Carbapenem resistant *Enterobacteriaceae.* Both the test are dependent on many variables like media components and thickness, incubation period and temperature, reading method, subjective variation in interpreting the result depending on the person reading the result etc. Though most of the parameters are standardised but few are difficult to standardise consistently which affects the reproducibility of phenotypic tests like mCIM and Kirby Bauer disk diffusion tests. However, mCIM is shown to have excellent reproducibility. In one of studies, validation study on mCIM was conducted to see its

reproducibility, where the mCIM test which was carried out by a lab was repeated by nine other labs. The result showed excellent reproducibility across laboratories [10-18]. Major limitation of our study was that we could not do genotypic characterisation of the carbapenem resistant isolates.

Conclusion

Our study has shown high resistance to carbapenems by production of carbapenemase enzymes and it is a matter of concern in Carbapenem resistant *Enterobacteriaceae* especially in *Klebsiella pneumoniae* and *Escherichia coli.* Therefore formulation of antibiotic policy and its strict implication is must to prevent the development of resistant in these species.

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