ABSTRACT

Introduction: In the era of increasing antimicrobial resistance, knowing the mechanism of carbapenem resistance can aid in choosing an apt drug for treatment of patients. There are only few studies estimating the burden of carbapenem resistance and determining the mechanism of resistance among the isolates in our locale.

Aim: To determine the prevalence of carbapenemase production by modified Carbapenem Inactivation Method (mCIM) among the Carbapenem Resistant Gram-Negative Bacteria (CRGNB)-Enterobacteriaceae and Pseudomonas aeruginosa isolated in our hospital.

Materials and Methods: This was a prospective observational study conducted in Department of Microbiology, KMCH, Coimbatore, Tamil Nadu, India during the period of June 2021 to August 2021. A total of 165 isolates of Enterobacteriaceae family and P. aeruginosa which were resistant to one of the carbapenems (imipenem, meropenem or ertapenem) by Vitek MIC testing were included in the study. All were subjected to mCIM and EDTA-modified CIM (eCIM) test and interpreted as per the CLSI M100 S31 guidelines.

Results: Among the 165 isolates, 130 (78.8%) were K. pneumoniae, 27 (16.4%) were other Enterobacteriaceae and eight (4.8%) were P. aeruginosa. Prevalence of mCIM positivity was 51.5% (85 isolates). Approximately, 98% of the mCIM positives (Carbapenemase Producing Gram-Negative Bacteria [CPGNB]) were eCIM positive indicating Metallo-Beta-Lactamase (MBL) production.

Conclusion: Performing mCIM in CRGNB is important in routine practice to identify CPGNBs. Due to very high prevalence of MBL among carbapenemase producers, it is advisable to choose ceftazidime avibactam plus aztreonam as the treatment option for CPGNB in our locale.

INTRODUCTION

Antimicrobial resistance is one of the most serious threats to humans, in the past decades. Increased prevalence of Multi Drug Resistant GNB (MDR-GNB) is becoming a greatest risk [1-3]. The carbapenems (belonging to the β-lactam group of antibacterial agents) are the common choice of treatment for emerging Extended Spectrum Beta Lactamase (ESBL) and AmpC enzyme producing gram-negative bacilli [4,5]. However, the prevalence of carbapenem resistance is also increasing in the current decade. Carbapenem resistance has been reported widely among Enterobacteriaceae particularly in Klebsiella pneumoniae and Escherichia coli besides P. aeruginosa spp. and Acinetobacter spp. [3,6,7].

Drugs used to treat CRGNB infections are partly based on the mechanism of resistance to carbapenem. Most common mechanism of carbapenem resistance in GNB is due to the production of carbapenem hydrolysing enzymes called carbapenemase which are either serine beta lactamase or MBL [8-10]. In general, beta lactamases inhibitors like avibactam, relebactam, vaborbactam, and monobactam are found effective in serine beta lactamase producers. Monobactam like aztreonam works against MBLs. However, when both enzymes are present in CRGNB, a combination of ceftazidime avibactam and aztreonam or cefiderocol is found effective and recommended by the Infectious Diseases Society of America guidelines [10,11]. In case of efflux pump-based resistance, alternative group of drugs are chosen as treatment options [10].

Among MBLs, IMP and VIM were discovered first. Later, the New Delhi MBL-1 (NDM-1) Enterobacteriaceae has emerged in Asian subcontinent and in European countries (Romania, Hungary, Spain, Denmark) and rapidly spread across the globe [8,12,13]. Hence, it is essential to know the data on the prevalence of carbapenem resistance, carbapenemase prevalence and type of carbapenemase produced by the bacteria to start an effective empirical as well as antibiotic susceptibility guided therapy. There are few data published about the existing prevalence of CPGNB in our locales [14,15]. Current study was performed to estimate the prevalence of carbapenemase production by a screening method—Modified CIM among the Carbapenem Resistant Enterobacteriaceae (CRE) and P. aeruginosa isolated from clinical samples from our hospital.

MATERIALS AND METHODS

This was a prospective observational study conducted in Department of Microbiology, KMCH, Coimbatore, Tamil Nadu, India on 165 carbapenem resistant gram-negative bacilli collected by convenient sampling method during the period of June 2021 to August 2021, from the clinical isolates after taking approval from the Institutional Ethical Committee. These isolates were identified as resistant or moderately susceptible to one or more of the carbapenems tested (imipenem, meropenem, ertapenem) by Vitek 2 automated AST method according to CLSI M100 S31 guidelines [16,17].
mCIM is done for suspected carbapenemase production in Enterobacteriaceae and P. aeruginosa; whereas eCIM is used together with mCIM to differentiate metallo b-lactamase from serine carbapenemase in Enterobacteriaceae. eCIM was tested for P. aeruginosa in the current study though it was not recommended by CLSI, but the data was excluded from statistical analysis. According to CLSI, mCIM screen test has a sensitivity and specificity of >99% for Enterobacterales and >97% and 100% for P. aeruginosa respectively. eCIM method demonstrated sensitivity and specificity of >95% and >92% for Enterobacterales, respectively [17].

**RESULTS**

Of the 165 isolates, 157 belonged to Enterobacteriaceae family and 8 were P. aeruginosa isolates. Majority of the CRE isolates were obtained from men (112 [68%]) compared to women (53 [32%]) and 75.32% of the patients were in the age group of 60-90 years. Distribution of study isolates across the sample is shown in [Table/Fig-3]. Most of isolates were obtained from in-patient wards 101 (61.2%) followed by ICU 43 (26.1%) and a very few percentages were from OPD 21 (12.7%).

[Table/Fig-3]: The split analysis of samples and different species studied with their mCIM and eCIM results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Klebsiella pneumoniae</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
<th>Enterobacter cloacae</th>
<th>Klebsiella aerogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates</td>
<td>mCIM +ve</td>
<td>eCIM +ve</td>
<td>mCIM +ve</td>
<td>eCIM +ve</td>
<td>mCIM +ve</td>
</tr>
<tr>
<td>Urine (72)</td>
<td>52</td>
<td>23 (42.2)</td>
<td>23</td>
<td>14 (62.5)</td>
<td>12 (91.2)</td>
</tr>
<tr>
<td>Respiratory sample* (35)</td>
<td>28</td>
<td>16 (57.1)</td>
<td>16</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Blood (30)</td>
<td>26</td>
<td>15 (57.7)</td>
<td>13</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Pus and fluids† (28)</td>
<td>24</td>
<td>10 (41.7)</td>
<td>10</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>64 (48.2)</td>
<td>62 (97)</td>
<td>20 (16.0)</td>
<td>16 (100)</td>
</tr>
</tbody>
</table>

* Respiratory samples were Sputum, Endotracheal aspirate and Bronchoalveolar lavage; † Fluids analysed were peritoneal, pleural and pericardial fluids

Taking different samples into consideration, K. pneumoniae isolated from blood and respiratory samples shows 57.7% carbapenemase production, each, in comparison to other samples, where the prevalence was ranging from 41.7-44.2%. E. coli were 50-100% carbapenemase producers across samples. Though mCIM positivity was highest among E. coli (16, 80%) in the current study, number of isolates was too small for generalisation of this data.

Prevalence of carbapenemase production by mCIM method was observed to be (85) 51.5% among the CRGNB included in this study. Among the 85 mCIM positive isolates, Enterobacteriaceae constituted 83 and the remaining 2 were P. aeruginosa. Among the 83 Enterobacteriaceae, 81 tested eCIM positive indicating presence of MBL enzyme. Approximately, 81 (97.6%) of CRE were MBL producers. Both the carbapenemase producing P. aeruginosa tested MBL positive. As the CIM methodology is not recommended for P. aeruginosa under CLSI guidelines, it was excluded from statistical analysis.

Distribution of carbapenemase in CRGNB was ranging from 42.3% to 56.7% in different samples, with higher prevalence being observed in blood samples followed by urine samples as shown in [Table/Fig-4]. Of the 165 isolates, 129 were resistant to all carbapenem tested by Vitek 2, while 36 were either moderately susceptible or susceptible to one or more carbapenem tested. Among these isolates, imipenem was the most common drug which showed moderate susceptibility or susceptible to one or more carbapenem tested. Among these isolates, imipenem was the most common drug which showed moderate susceptibility or susceptible to one or more carbapenem tested. Among these isolates, imipenem was the most common drug which showed moderate susceptibility or susceptible to one or more carbapenem tested.
All isolates which were mCIM positive (85 isolates) were resistant to both ertapenem and meropenem whereas 8.2% (7 isolates) were moderately susceptible to imipenem. Among the 35 isolates, which were moderately susceptible to imipenem, only four were moderately susceptible to either one or two of the other carbapenemase tested. All four isolates tested mCIM negative.

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of CGGNB</th>
<th>No. of mCIM positive isolates N (%)</th>
<th>No. of eCIM positive isolates N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>72</td>
<td>38 (52.8)</td>
<td>38 (52.8)</td>
</tr>
<tr>
<td>Respiratory sample</td>
<td>35</td>
<td>18 (51.4)</td>
<td>18 (51.4)</td>
</tr>
<tr>
<td>Blood</td>
<td>30</td>
<td>17 (56.7)</td>
<td>15 (50.0)</td>
</tr>
<tr>
<td>Pus and fluids</td>
<td>28</td>
<td>12 (42.3)</td>
<td>12 (42.3)</td>
</tr>
<tr>
<td>Total</td>
<td>165</td>
<td>85 (51.5)</td>
<td>83 (50.3)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Carbapenem hydrolysing beta lactamases are classified into Class A (KPC, IMI) and Class D (OXA-48, OXA-181, OXA-23, OXA-40, OXA-58) serine beta lactamases and Class B (IMP, VIM, NDM) MBLs. KPC followed by OXA is the most common variety found in western countries. Based on the past studies, in Asian subcontinent MBL especially NDM-1 and OXA 48 are the most common ones [9,18].

NDM was first identified in Sweden from an Indian returnee [19]. Later, studies showed that this was the most common gene in India, Pakistan and Bangladesh regions [12]. Study by Walsh TR et al., showed the magnitude of existence of NDM bugs in Indian environment [20]. In 2015, Kazi M et al., demonstrated the co-existence of NDM, OXA 48 and VIM genes in same bacteria [18]. Prevalence of carbapenemase production especially MBLs (51.5% & 97.6%) in current study was similar to other studies in different parts of the country except for Maharashtra study (98% mCIM positivity) [3]. Study in aiIMS, New Delhi 2019 stated carbapenemase prevalence as 65% in Enterobacteriales by Modified Hodge test and PCR [21]. A study in Lucknow 2014, recorded 100% prevalence of NDM with or without co-existing AmpC beta lactamase genes in all the carbapenem resistant Enterobacteriales [22]. Prevalence of carbapenemase by mCIM was 45.09% in Tumkur study in Karnataka [23]. Studies in Coimbatore region of Tamil Nadu had observed prevalence of 73.33% in 2011-2015 and 82% in 2017. However, these studies have focused on isolates from specific samples in patients with certain comorbidities. Some of these studies were detecting only a specific group of carbapenemase producing genes by PCR [14,15]. Comparative prevalence reports of carbapenemase in various regions of India are collated in [Table/Fig-7] [3,14,15,21-27].

AMR surveillance network report states that prevalence of carbapenem resistance was 33% in Enterobacteriales and MBL genes were found in 25% of these isolates on an average. A relatively high prevalence of NDM was observed in certain regions of the country. Resistance has increased averagely by 4.9%, 4.4% and 6% in *E. coli*, *K. pneumoniae* and *Enterobacter species* over 5 years (2016-2020) in India, respectively [7].

On analysing the antibiogram pattern of carbapenem, among mCIM positive and negative isolates, it was found that isolates which were moderately susceptible to imipenem were less likely to be carbapenemase producers compared to resistant ones. It was observed that all mCIM positive isolates were resistant to ertapenem and meropenem, by Vitek MIC determination. This observation shows a potential for using antibiogram pattern in helping with empirical choice of drug. However, studies with larger sample size, aimed at confirming this observation is required in future.

**Limitation(s)**

eCIM gives a positive result irrespective of presence of serine carbapenemase genes along with MBL. Hence, this method can’t differentiate presence of co-existence of other carbapenemase genes along with MBL, from presence of only MBL genes. Due to disparity in the distribution of number of isolates, observations made using this variable requires further study to confirm the findings.

**CONCLUSION(S)**

It is advisable to perform mCIM in carbapenem resistant isolates as a routine, to decide on the best choice of empirical antibiotic for treatment. Very high prevalence of MBLs suggested by eCIM results, show that ceftazidime avibactam plus aztreonam would be the best option for mCIM positive isolates in clinical practice in this study region.
REFERENCES


