Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Occurrence and types of carbapenamase enzymes amongst enterobacterales and *Pseudomonas spp*. using automated phenotypic method

Sumit Sonaba Chavan[1], **Kalpana Mohan Angadi**[1], **Rajal Pranav Dave**[2]*

[1]*Dept. of Microbiology, Symbiosis Medical College for Women (SMCW), & Symbiosis University Hospital & Research Centre, Pune, Maharashtra, India*
[2]*Green array Genomic Research & Solution Pvt., Pune, Maharashtra, India*

ARTICLE INFO

Article history:
Received 05-05-2024
Accepted 24-05-2024
Available online 22-06-2024

Keywords:
Infection
enterobacterial
Carbapenemase

ABSTRACT

Background: The continuous rise of Carbapenem Resistant Organisms, considered as one of demanding public health issue. Carbapenemase producing organisms (CPO) can be detected by various molecular and phenotypic methods. Although molecular method shows higher accuracy, but is limited by relatively complex procedure and expensive regents. Thus, phenotypic methods considered as routine CPO detection method. Automated system Phoenix M50 (Becton & Dickinson) CARBA panels, provides the advantage of simultaneous identification and characterization of carbapenemase enzymes.
Materials and Methods: A retrospective study was conducted for various clinical samples received at a tertiary care center in Western Maharashtra during a period of one year. The bacterial identification and antimicrobial susceptibility testing performed by automated system Phoenix M50 (Becton & Dickinson) CARBA panels. Isolates which flagged as carbapenemase producers were confirmed using modified carbapenemase inactivation method (mCIM) as a part of protocol.
Results: Among all isolates of enterobacterial and *Pseudomonas spp*. 32% were carbapenemase producers. ***E-coli*** identified as predominant strain. Class B MBL was 46.4 % of isolates, consisting of *E-coli* appeared at 40% followed by *P.aeruginosa* at 35.5%. Class D beta-lactamase the second, the majority found in K.pneumoniae at 27.23%, followed by *E-coli* at 30 %. For Class A, *P.aeruginosa* 56 % followed by 26 % (n=67) isolates found in K.pneumoniae. The most isolates were Urine 16.6% followed by Pus 7.29 %, Respiratory 4% and blood 4%.
Conclusions: A high occurrence of carbapenemase producers observed in the study is concerning. Moreover, most of them belonged to Class B MBLs, which demonstrated the need antibiotic stewardship and hospital infection control strategies to prevent their further spread.

1. Background

The emergence and continuous transmission of multi Drug resistant (MDR) microorganisms have become public health issue. On a most common account infection caused by Multi Drug resistant organisms, which has significant impacts on patient's outcome for community and hospital acquired infection. Centre for Disease and Research (CDC) defines Carbapenem resistant bacteria as resistant to one more carbapenem antibiotic or carbapenamase producer enzyme and classifies them into high risk category for public health and thus emphasizing on addressing issue as an urgent threat.[1] Since many years, Carbapenems considered as most effective antibiotic to treat hospital acquired infections caused by Multi Drug resistant organisms, including extended spectrum β- lactamases producers. Currently emergence of Carbapenem resistant organisms

* *Corresponding author*.
E-mail address: rajaldave12@gmail.com (R. P. Dave).

https://doi.org/10.18231/j.ijmmtd.2024.025

which endow broad resistant to most β-lactam antibiotics exclusively "last –line" antibiotics.

The most predominant mechanism includes plasmid mediated, carbapenem hydrolysing enzyme production. Carbapenemase producing bacteria can be classify on the basis of their conferring different enzymes, Carbapenemases, is one of the issuable enzymes after first identified in strain – K.pneumoniae carbapenamase which is (KPC), Oxacillinase which is OXA (Oxacillin), New-Delhi Metallo –β lactamase (MBL) -NDM and Verona Integron- mediated Metallo – β- lactamase, VIM (Verona Integron-encoded Metallo-beta –lactamase). As Carbapenem resistant organisms incidences rises, often times infection being treated by certain groups of antibiotics like Tigecycline, fosfomycin and Colistin before onrush of newer agents like ceftazidime/avibactam, meropenem/vaborbactam and cefiderocol. Carbapenem resistant genes transmitted by plasmid mediated. So as the resistance to these antibiotics has been rises, their transmission is difficult to control (Figure 1).

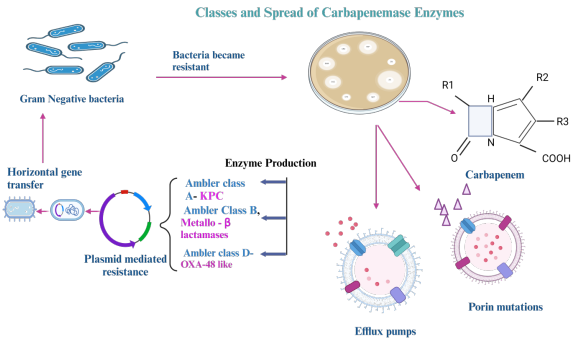

Figure 1: Classes and spread of carbapenemase enzymes [2]

BD phoenix N500 panel (CPO panel) detects as well as classifies carbapenemase enzymes based on organism growth in presence of Meropenem, Temlocillin, Doripenem and Cloxacillin drugs, alone and in combination with various beta-lactamase inhibitors and chelators. Panel classifies carbapenemase into different classes as per Ambler classification for beta-lactam enzymes. Ambler Class A is broad group of beta lactamases encoded by plasmids or chromosomes. Class A includes enzymes NMC, KPC, IMI etc. are commonly encountered in the enterobactericea group. It's rarely observed in *Pseudomonas spp*. commonly used newer combinations utilizing beta-lactamase inhibitors shown to be protective against class A and some of class D carbapenamase producing isolates.

Ambler Class B is metallobetalactamase enzymes, which includes VIM, GIM, and NDM type enzymes. It's commonly encountered in *E-coli*, Klebsiella spp, *P.aeruginosa* and Acinetobacter spp. Metallobetalactamase are considered resistant to even newer beta-lactam and beta-lactamase inhibitor combinations which are protective against other classes of carbapenamases. Class D includes OXA-48 type and more commonly observed in *Acinetobacter spp*.[3] Class C is Cephalosporinase group producing Amp C enzymes which usually confers resistance to Cephalosprins and beta lactam / beta lactamase inhibitor combinations However, certain Amp C enzymes can hydrolyze one or more carbapenems. This is chromosomally mediated resistance. Amp C producing organisms may develop resistance to carbapenem group through porin channel or efflux pump formation.[3–5] Considering multidrug resistance associated with this organism, clinicians mostly resort to synergistic combination of drugs as treatment modality.

Carbapenamase resistant organisms can be detected by various molecular and phenotypic methods. Although molecular method shows higher accuracy, their utility in diagnostic laboratories is limited by relatively complex procedure, expensive regents and high turnaround time.

Thus phenotypic methods are looked up to as possible routine carbapenamase detection method in clinical microbiology laboratories. Phenotypic methods are growth based which include modified Hodge test, modified carbapenem inactivation method etc. Phenotypic methods utilizing carbapenem hydrolysis includes Carba NP test, MALDI-TOF (Matrix-Assisted Laser Desorption Ionization –Time of Flight Mass Spectrometry) etc.

2. Materials and Methods

A retrospective study was conducted for various clinical samples received at a tertiary care center in Western Maharashtra during a period of one year (Jan 2023-Dec 2023). The bacterial identification and antimicrobial susceptibility testing performed by automated system Phoenix M50 (Becton & Dickinson) CARBA panels. Results interpreted as per CLSI (Clinical and Laboratory Standard Institute) guidelines.[6] Here sterility check surveillance specimens were excluded for this study. These false positive carbapenamase nonproducers may include CRO by mechanism other than the carbapenamase enzyme. Thus they were excluded from the study.

Isolates of enterobacteriaceae group and *P.aeruginosa* showing resistance to one or more carbapenem antibiotic (Minimum inhibitory concentration of Ertapenem >1μg/mL, Minimum inhibitory concentration of Meropenem and Imipenem 2-4 μg/mL) and reported as carbapenem producer by phoenix automated system were included in the study. All isolates flagged as carbapenemase producers were further confirmed using modified carbapenemase inactivation method (mCIM) as a part of protocol. We studied dissemination of different classes of carbapenemase among clinical isolates provided by CARBA panels phenotypically. Figures 1, 2, 3, 4, 5, 6 and 7 made in BioRender.com to explain mode of action of antimicrobial and new antimicrobial approaches to prevent

increase of antimicrobial respectively.[7]

2.1. Confirmation of carbapenemase producer enterobacteriaceae (CPE)

For that modified carbapenemase Inactivation method (mCIM) was performed. A two and four loopful of freshly subculture growth of enterobacteriaceae and pseudomonas species diluted in 2 ml TSB (Trypticase soya broth) broth in a test tube. Broth has been vortex for few minutes. Meropenem disc (10μg, Hi-media) immersed into the test tube followed by incubation for four hours. After completion of incubation, disc was removed by pressing against side of test tube to remove liquid in excess. Lawn culture using ATCC (American Type culture collection) strain of *E-coli* was prepared on Mueller –Hinton Agar. Meropenem disc taken out of test tube placed on agar plate and the plate incubated at 37 °C for 18-24 hrs.[6,8] An organisms showing zone diameter of less than 19 mm or presence of growth within the zone were confirmed as carbapenamase producer and their antibiotic susceptibility result was analyzed (Figure 7).

3. Results

Total 1123 clinical specimens received from Jan 2023 – Dec 2023 time period. Out of 1123, 675 (60.10%) specimens found Carbapenem Resistant as well as multi-drug resistant organisms (Figure 2). Among all isolates of enterobacterial and *Pseudomonas spp*. 32% (211/675) were found carbapenemase producers. *E-coli* stood as most predominant strain. Most of the CPO isolates were obtained from critical care unit patients. Around 240 isolates flagged positive as CPO by Phoenix BD system, 22 (9%) found negative by mCIM method. Finally 211 isolates considered for this study.

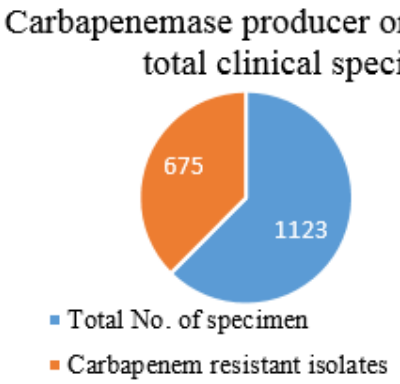

Figure 2: Isolation of Carbapenem resistant organisms from clinical specimens

3.1. Isolate wise class distribution

Out of 675 clinical samples, For Class B MBL, 257 specimens were the most commonest class account of isolates, consisting primarily of *E-coli* at 46.4% (n=39) closely followed by *P.aeruginosa* at 36% (n=21) and *K.pneumoniae* at 16 % (n=40). Class D beta-lactamase appeared as the second commonest class. The majority found in *E-coli* at 30% (n=25) followed by *K.pneumoniae* at 27.23 % (n=70). For Class A, *P.aeruginosa* 56 % (n=33) followed by 26 % (n=67) isolates found in *K.pneumoniae* (Figure 4). Undetermined class were identified as potential carbapenemase producers by CARBA panels without further classification. Apart from *E-coli*, *K.pneumoniae* and *P.aeruginosa*, other species of Klebsiella, Proteus spp., *Providentia spp*., *Citrobacter spp*., and *S.typhi* also isolated as a carbapenemase producer (Figure 3).

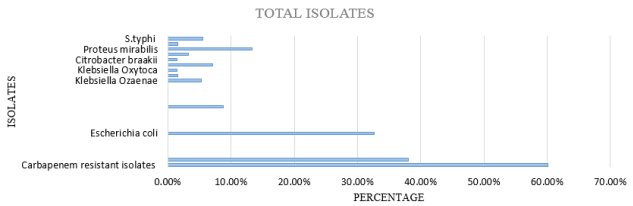

Figure 3: List of Carbapenamase isolates

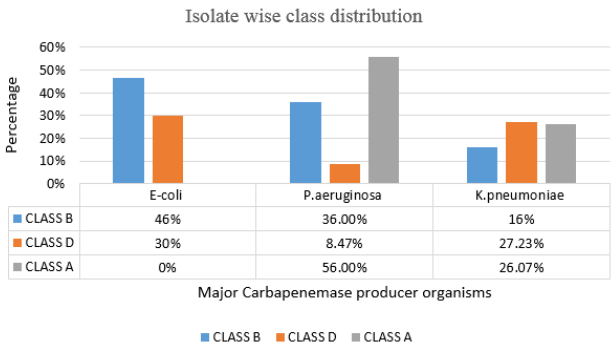

Figure 4: Class wise distribution of major carbapenemase producer isolates

The most common CPO source was Urine specimens 16.6% (n=107) followed by Pus 7.29% (n=49), respiratory (sputum, endotracheal secretions, Broncho alveolar fluid) 4% (n=26), Blood (and body fluids) 4% (n=29) (Figure 5). Overall resistance to Ciprofloxacin was 93.9% to Levofloxacin was 97.4% and to Piperacillin-tazobactam was 90.3%. Among aminoglycosides, resistance to Amikacin was 61.9% and to Gentamicin was 72.6%. Colistin and tigecycline resistance were observed in 15.3% and 14.2% patients respectively (Figure 8).

Among Class B carbapenamase, Coproduction of ESBL (Extended spectrum Beta Lactamase) was observed in

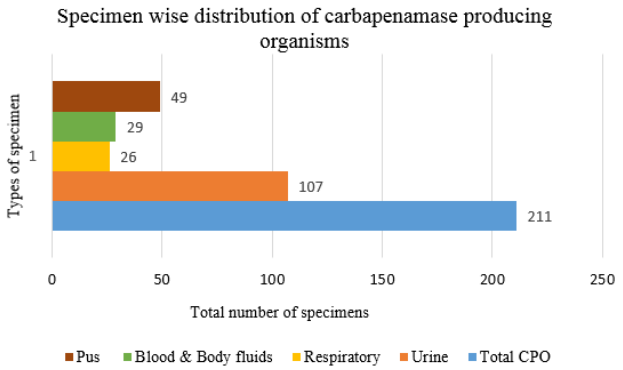

Figure 5: Specimen wise distribution of carbapenemase producing organisms

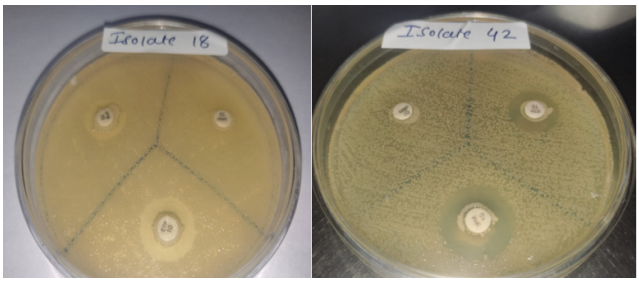

Figure 7: Modified carbapenemase Inactivation method test result

83% and 86% of isolates of *E-coli* and *Klebsiella species* respectively. While, Amp C production was seen in 55% and 50% isolates of *E-coli* and *Klebsiella species* respectively. Most Class B isolates of *E-coli* were multi-drug resistant. They showed 100% resistant to all antimicrobials except Amikacin, Gentamicin, Tigecycline and Colistin. Amikacin and Gentamicin showed resistance in 38% and 55% cases while no resistance was observed for Tigecycline and Colistin (Figure 6).

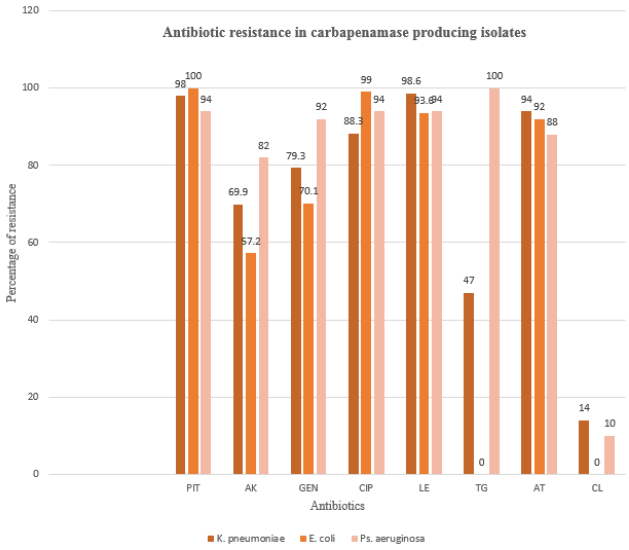

Figure 6: Percentage of antibiotic resistance in carbapenamase producing isolates

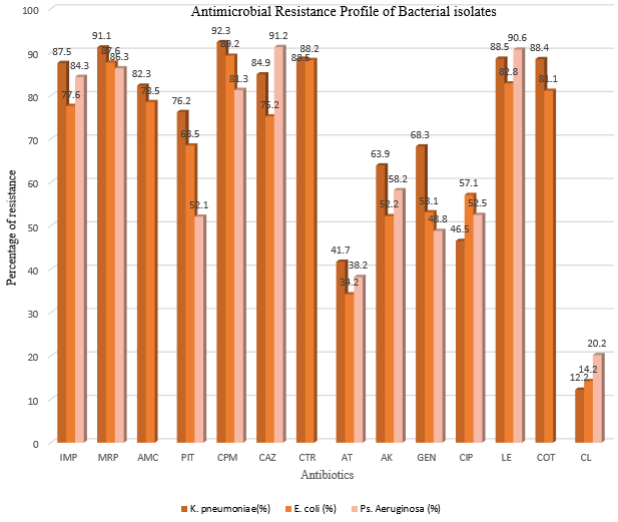

Figure 8: Antimicrobial resistance profiles of bacterial Isolates

4. Discussion

Monobactams such as Aztreonem are stable against carbapenem resistant organism and need to be added to newer combinations for management of infections caused by MBL producing organism. This regimen also covers ESBL and Amp C class of beta- lactamases which are

Table 1: Antimicrobial resistance profiles of bacterial Isolates

Antibiotic	K. pneumoniae %	E. coli %	Ps. Aeruginosa %
PIT	98	100	94
AK	69.9	57.2	82
GEN	79.3	70.1	92
CIP	88.3	99	94
LE	98.6	93.6	94
TG	47	0	100
AT	94	92	88
CL	14	0	10

*PIT- Piperacillin – tazobactam, AK-Amikacin, GEN- Gentamycin, CIP – Ciprofloxacin, LE – Levofloxacin, TG-Tigecycline, AT- Aztreonem, CL-Colistin

Table 2: Antimicrobial resistance profiles of bacterial isolates

Antimicrobial agents	*K.pneumoniae* (%) R	*E-Coli* (%) R	Ps.aeruginopsa (%) R
Imipenem	87.5	77.6	84.3
Meropenem	91.1	87.6	86.3
Colistin	12.2	14.2	20.2
Amoxicillin-clavulanic	82.3	78.5	86.4
Piperacillin - tazobactam	76.2	68.5	52.1
Cefepime	92.3	89.2	81.3
Ceftazidime	84.9	75.2	91.2
Ceftriaxone	88.5	88.2	94.8
Aztreonem	41.7	34.2	38.2
Amikacin	63.9	52.2	58.2
Gentamycin	68.3	53.1	48.8
Ciprofloxacin	46.5	57.1	52.5
Levofloxacin	88.5	82.8	90.6
Trimethoprim-Sulfamethoxazole	88.4	81.1	89.2

often coproduced with MBLs.[9] Early identification of type of cabapenamase has direct therapeutic implications especially in critical cases of multi drug and pan drug resistant infections. Awareness on pattern of prevalent CPO is important for effective implementation of antibiotic stewardship and infection control measures. Hospitals isolates patient infected with CRE to prevent bug transmission especially in critical care settings based on availability of resources. Class B MBLs were the most common class accounting for 47% of isolates, consisting primarily of K. pneumoniae at 40% closely followed by *E-coli* at 39%, *P.aeruginosa* at 9%, Acinetobacter species at 6%, and Enterobacter species at 6%.

Overall Class B isolates exhibited complete sensitivity to Colistin (100%), Tigecycline 89% followed by Amikacin 64%, Minocycline 47%, and Gentamicin 42%. The susceptibility pattern for other antibiotics includes Meropenem 13%, Imipenem 20%, Piperacillin-tazobactam 13%, Ceftazidime-avibactam 13%, Ciprofloxacin 13%, Cotrimoxazole 40%, Nitrofurantoin 58% (in urinary isolates) and Fosfomycin 80% (in urinary *E-coli*).

Class D beta-lactamase appeared as the second commonest class at 36%, the majority found in K. pneumoniae at 70%, followed by *E-coli* at 25%, and other Enterobacter species at 5%.

A surprisingly higher degree of resistance existed in the Class D isolates than that in Class B. Class D isolates exhibited poor sensitivity to Amikacin 18% and Gentamicin 11%. Colistin was sensitive at 90% closely followed by Tigecycline at 86%. Only three isolates were of Class A (3%) which comprises two K. pneumoniae and one *P.aeruginosa*.

This study observed 14% of isolates flagged by the system as carbapenemase producers, however, the panel could not determine the class of carbapenemase enzyme (Unclassified) possibly because of multiple carbapenemase productions or heterogeneous bacterial populations or the presence of other mechanisms of CR resistance with or without the carbapenemase production. An evaluation study on N500 panels has shown good sensitivity and specificity for detection and classification into carbapenamase type of around 98% and 100% respectively amongst carbapenamase producing organisms. However specificity was as low as 32% for non carbapenamase producing CREs. Most of the false positives were misidentified as untyped carbapenamase producer. The study suggests additional confirmatory method for carbapenamase classification.[10] A spectrum of carbapenemase enzymes in western countries differs substantially from that observed in India.

A surveillance study by ICMR for the year 2021 demonstrates a worrisome increasing trend in carbapenem resistance among important gram-negative pathogens obtained from numerous hospitals across India. In comparison with data from 2016, a study reports a rise in carbapenem-resistant *E-coli* and *K.pneumoniae* from 14% to 36% and 35% to 57% respectively over the previous four years. The most prevalent beta-lactamase genes associated with *E-coli* were Class B - IMP (37%) and NDM (31%), followed by Class D – OXA (30%). For K. Pneumoniae, Class D (OXA-48-like) followed by Class B (NDM) were most common. The report put up an alarm for exceptionally high NDM prevalence in the Pseudomonas isolates.[11]

While the northeast region is known for a lesser number of MDR infections in comparison with other parts of India owing to its scarce population, recent studies highlight the rapid dissemination of resistant gram-negative in this part of the nation. A recent study from Mizoram, a northeast area of India showed a CPO prevalence of around 11% among Enterobacterales.[3] Another study conducted in Asam documented the prevalence of CREs to be 18.9%.[12] Thus all reviews and this study itself suggesting increase of drug resistant strongly require new antimicrobial which be alternate of antibiotics.

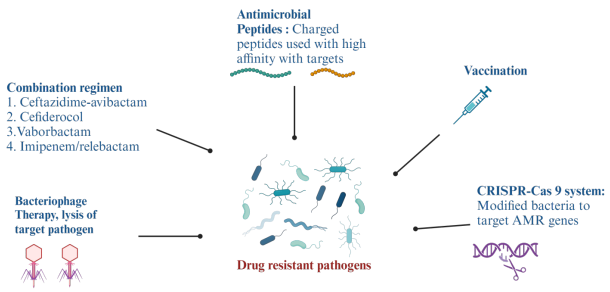

Figure 9: Possible therapeutic options for treatment of infection by carbapenem resistant bacteria[13]

To support antibiotic stewardship program and implementation of new infection control strategies, recently many clinical trials and case studies has shown that successfully treated patients through application of potent lytic phages or engineered phage with combination of antibiotics, patients who had extensively drug resistant bacterial infection and antibiotic failed to treat (Figure 9). So by developing new antibiotics with advance techniques can be possible approach to prevent antibiotic pandemic and secure the public health. [2,14]

5. Limitations of the Study

Our study carries several limitations which include lack of clinical information, overrepresentation of tertiary care centers as the majority of the samples were from the same, etc. Another major limitation of our study was the lack of genotypic confirmation of carbapenemase groups as well as test comparison with other tests. Analysis was done on basis of data provided by novel BD phoenix M50 panels which carry several flaws. Various studies all across the world have evaluated the performance of the BD CPO panel. A majority of studies demonstrate good sensitivity for carbapenemase detection with modest specificity.

Although the system correctly classifies carbapenemase enzymes into corresponding ambler classes, a good proportion of isolates remains unclassified. Possible reasons for the unclassified status of an isolate include the presence of multiple resistance mechanisms, heterogeneous resistance, and improper growth. Thus in isolates with more than one Carbapenamase type system fails to simultaneously identify both the types. These studies suggest the use of additional confirmatory methods to lower the false positives mainly for the unclassified CPO group. [15]

A previous study evaluated the efficiency of the CPO panel to detect and classify carbapenemase by using multiplex PCR as a reference method. It observed detection sensitivity and specificity of 89.7% and 83.5% respectively. The ability of the panel to correctly identify and classify depends upon the type of clinical isolate and ambler class. The CPO panel correctly classified class B and class D in 82.9% and 89.8% of the clinical isolates tested. A study doesn't demonstrate any misclassification of the Ambler group. [16]

Phenotypic tests are potential screening methods to identify CPO isolates before further application of expensive molecular assays.

6. Conclusion

The study showed a high burden of antibiotic resistance amongst CPOs and their spread always remain concern of public health. In today's era of advanced technologies, it's crucial for laboratories to maintain consistent surveillance and infection control strategies to prevent antimicrobial resistance. In CPOs, it's vital to detect resistance mechanism for providing precise antimicrobial therapy. There are many other therapeutic options need to try and implement in clinical microbiology laboratories.

7. Data Availability

Data are available within the article.

8. Conflict of Interest

Authors declares there is no conflict to publish this paper.

9. Source of Funding

None.

Acknowledgements

This research work supported by Symbiosis Medical College for Women (SMCW) & Symbiosis University Hospital & Research Centre (SUHRC), Pune. We are thankful to Dr. Kalpana Angadi for review and approval of this study.

References

1. Ramanathan YV, Venkatasubramanian R, Nambi PS, Ramabathiran M, Venkataraman R, Thirunarayan MA, et al. Carbapenem-resistant enterobacteriaceae screening: A core infection control measure for critical care unit in India? Indian. *Indian J Med Microbiol*. 2018;36(4):572–6.
2. Patel DR, Bhartiya SK, Kumar R, Shukla VK, Nath G. Use of customized bacteriophages in the treatment of chronic nonhealing wounds: a prospective study. *Int J Low Extrem Wounds*. 2021;20(1):37–46.
3. Ralte VSC, Loganathan A, Manohar P, Sailo CV, Sanga Z, Ralte L, et al. The Emergence of Carbapenem-Resistant Gram-Negative Bacteria in Mizoram, Northeast India. *Microbiol Res*. 2022;13(3):342–9.
4. Codjoe FS, Donkor ES. Carbapenem Resistance: A review. *Med Sci*. 2017;6(1):1. doi:10.3390/medsci6010001.
5. Ambler RP, Coulson AF, Frère JM, Ghuysen JM, Joris B, Forsman M, et al. A standard numbering scheme for the class A beta-lactamases. *Biochem J*. 1991;276(Pt 1):269–70.
6. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 34th ed. CLSI supplement M100. CLSI; 2024. Available from: https://clsi.org/standards/products/microbiology/documents/m100/.
7. "Classes and Spread of Carbapenemase Enzymes", by BioRender.com (2024) 49 Spadina Ave. Suite 200 Toronto ON M5V 2J1 Canada, www.biorender.com. Available from: https://app.biorender.com/biorender-templates.
8. Ong CH, Ratnayake L, Ang MLT, Lin RTP, Chan DSG. Diagnostic accuracy of BD Phoenix CPO Detect for carbapenemase production in 190 Enterobacteriaceae isolates. *J Clin Microbiol*. 2018;56(12):1043–1061.
9. Antimicrobial Therapy Inc. The Sanford Guide to Antimicrobial Therapy. 53rd Edn. Antimicrobial Therapy, Inc; 2023.
10. Cho H, Kim JO, Choi JE, Lee H, Heo W, Cha YJ. Performance evaluation of automated BD Phoenix NMIC-500 panel for carbapenemase detection in carbapenem-resistant and carbapenem-susceptible Enterobacterales. *J Microbiol Methods*. 2020;177:106042. doi:10.1016/j.mimet.2020.106042.
11. Indian Council of Medical Research (ICMR). AMR surveillance Network, Jan 2021- Dec 2021.

12. Sharma M, Chetia P, Puzari M, Neog N, Borah A. Menace to the Ultimate Antimicrobials among Common Enterobacteriaceae Clinical Isolates in Part of North-East India. *BioRxiv*. 2019;p. 610923. doi:10.2174/2211352519999210128174853.
13. Ong CH, Ratnayake L, Ang MLT, Lin RTP, Chan DSG. Diagnostic Accuracy of BD Phoenix CPO Detect for Carbapenemase Production in 190 Enterobacteriaceae Isolates. *J Clin Microbiol*. 2018;56(12):e01043–18. doi:10.1128/JCM.01043-18.
14. Johri A, Johri P, Hoyle N, Nadareishvili L, Pipia L, Nizharadze D, et al. Case report: Successful treatment of recurrent E. coli infection with bacteriophage therapy for patient suffering from chronic bacterial prostatitis. *Front Pharmacol*. 2023;14:1243824. doi:10.3389/fphar.2023.1243824.
15. Thomson G, Turner D, Brasso W, Kircher S, Guillet T, Thomson K, et al. High-Stringency Evaluation of the Automated BD Phoenix CPO Detect and Rapidec Carba NP Tests for Detection and Classification of Carbapenemases. *J Clin Microbiol*. 2017;55(12):3437–43.
16. Albichr IS, Anantharajah A, Dodémont M, Hallin M, Verroken A, Rodriguez-Villalobos H, et al. Evaluation of the automated BD Phoenix CPO Detect test for detection and classification of carbapenemases in Gram negatives. *Diagn Microbiol Infect Dis*. 2009;96(2):114911. doi:10.1016/j.diagmicrobio.2019.114911.

Author biography

Sumit Sonaba Chavan, Assistant Professor https://orcid.org/0000-0001-7123-7847

Kalpana Mohan Angadi, Professor & HOD https://orcid.org/0000-0001-5588-7524

Rajal Pranav Dave, Senior Scientific Officer https://orcid.org/0000-0001-8587-8979

Cite this article: Chavan SS, Angadi KM, Dave RP. Occurrence and types of carbapenamase enzymes amongst enterobacterales and *Pseudomonas spp*. using automated phenotypic method. *IP Int J Med Microbiol Trop Dis* 2024;10(2):138-144.