I J R B A T, Issue (XIII) Volume (I) Jan 2025: 85-91
A Double-Blind Peer Reviewed & Refereed Journal
OPEN ACCESS
e-ISSN 2347 – 517X
Review Article
INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
 www.ijrbat.in

CARBAPENEMASE PRODUCTION & DETECTION IN GRAM NEGATIVE BACTERIA

Shalini Pandey[1], Seema Nimbarte[2], P.R.Bhandari[3] and Y.S.Thakar[4]
[1,2,3]Department of Microbiology, Sevadal Mahila Mahavidalaya,Nagpur
[4]Director of Vishakha Clinical Microbiology Laboratory, INDIA
Corresponding Email :- yranu9767@gmail.com

Communicated :15.11.2024 | Revision: 25.11.2024 & 15.12.2024 Accepted: 26.12.2024 | Published: 30.01.2025

ABSTRACT:
The global spread of multidrug resistant gram negative bacteria is quite well evident. Carbapenem class of antibiotics are favoured treatment options for severe and life threatening infections due to gram negative bacteria especially by those belonging to *Enterobacteriaceae, Pseudomonas, Acinetobacter* and other non-fermenting Gram negative bacteria. However, these organisms have started developing resistance to carbapenem antibiotics mainly by production of carbapenemase enzymes which have multiple genetic determinants viz *Klebsiella pneumonia* Carbapenemase (KPC), New Delhi Metallo-beta-lactamases (NDM), OXA, IPM, VIM etc. and occasionally by Porin mutation and efflux mechanisms. This results in limited choice of treatment. The increasing prevalence of carbapenemase producing organisms (CPO) is a matter of concern; hence detection of CPOs by finding at carbapenemase production in them and eradicating them with proper antimicrobial therapy is essential. There are many genetic loci in CPOs for carbapenemase production. The CPOs can be detected by different phenotypic methods as well as genotypically by PCR test. There is no single phenotypic test which is rapid, simple and can detect all types of Carbapenemases. However, they are easy to perform, accurate, economical and feasible in moderately equipped laboratories. In view of rapid increase in drug resistance among bacteria it is essential to detect carbapenemase production to advocate proper therapy to eradicate the resistant bugs and rationalize the treatment.

Keywords: - Carbapenemase, Enteriobacteriaceae , KPC, NDM, OXA.

INTRODUCTION :

Carbapenems are broad spectrum antimicrobial agents which comprise of Imipenem, Meropenem, Doripenem And Ertapenem. They are stable against wide range of beta lactamases produced by organisms, hence very effective in treatment. They are commonly used in intra abdominal infections, complicated urinary tract infections, hospital acquired or ventilator associated pneumonia and blood stream infections. Due to the safety, efficacy and broad spectrum of activity they are favoured drugs in life threating infections. However, increasing resistance is seen against these carbapenem antimicrobial agents. Earlier studied that 31.77% of *Enterobaceriaceae* clinical isolates were Carbapenem resistant, with *Klebsiella pneumonia* (63%) & *E.coli* (19%) being the predominant organisms. This creates lot of problems in management of patients as very few drugs will be available to treat such carbapenem resistant and multi drug resistant pathogens. Therefore understanding the mechanism of resistance is very important for managing such infections. In the following text, the mechanism of resistance of organisms to carbapenem drugs is discussed along with the methods of their detection.

Mechanisms of resistance:

Different mechanisms operate to confer the resistance to carbapenem which include
Enzyme destruction :- Carbapenemases
Altered targets :- DNA gyrase
Porin alterations: - Altered permeability or efflux of antimicrobial agent from bacterial cell.

The commonest and effective mechanism of resistance is carbapenemase production. The genes responsible for carbapenemase production

could be chromosomal or acquired through plasmids, integrons or trasposons.

Common carbapenem resistant organisms (CRO) are Carbapenem resistant *Enterobacteriaceae* (CRE) and if it produces carbapenemase it is termed as carbapenemase producing Carbapenem resistant *Enteriobacteriaceae* (CP-CRE). Likewise other common organisms that are carbapenemase producing including *pseudomonas aeruginosa* (CPPA) and carbapenemase producing *Acienetobacter baumanii* (CPAB).

The increased concern about carbapenemase producing organisms is for various reasons. The treatment options are limited. The exchange of resistance genes occur amongst pathogenic bacteria as well as environmental bacteria. The infections are associated with high economic cost as well as increase mortality.

Carbapenemases types:

Carbapenemases are classified as per the ambler classification scheme and belong to ambler class A,B and D.

Class A & D are serine Carbapenemases while class B is Metallo-beta-lactamase (MBL) *Klebshiella pneumonia* carbapenemase (KPC) belong to class A commonly found in United States more often seen in *Enteriobacteriaceae* and occasionally in *Pseudomonas aeruginosa or Acienetrobacter baumanii*

Class B are Metallo-beta-lactamases (MBLs) which require zinc ions. Chelators like EDTA inhibit activity of MBLs. New Delhi Metallo-beta-lactamse are (NDM), Verona integrin-encoded Metallo-beta-lactamse (VIM) and Imipenem resistant Phenotype (IMP) are common MBLs. Group D carbapenemase comprise of OXA Carbapenemases. They have high hydrolytic activity against Oxacillin. OXA-48 are common in *Enteriobacteriaceae.* OXA 23,24,51,58 are common in *Acienetrobac*ter OXA-48like is not seen in non – fermenters.

Initiallyit was observed that Carbapenemases were only chromosomally mediated. But later on they have been observed in plasmids as well as both in chromosomes and plasmids. This horizontal transfer between different bacterial species &genera has resulted in more severe spread of resistance.

Class A enzymes degrade penicillin, Cephalosporin's as well as carbapenem. Class B display activity against all Beta-lactam antibiotics except monobactam (Aztreonam). They are not inhibited by clavulanic acid but can be inhibited by EDTA. The OXA-48 has less activity against carbapenem and cephalosporin's, but has high activity against penicillin. The most frequent carbapenemases throughout the world are MBLs (IMP, VIM & NDM), KPC & OXA and they are the predominant resistance mechanisms among the clinical isolates. The diverse range of carbapenemase enzyme makes them difficult in their detection.

The detection of carbapenemase can be done by the phenotypic and genotypic methods

The phenotypic assay:

i. Growth based assay: They measure the growth of bacteria in presence of antibiotic, e.g. Modified Hodge test (MHT), modified Carbapenem Inactivation method (mCIM). EDTA Carbapenem Inactivation method (eCIM)
ii. Hydrolysis methods: They detect the product of hydrolysis catalysed by Carabapenem enzymes e.g. carba NP and Matrix Assisted Laser Desorption–Ionization Time Of Flight Mass Spectrometry. (MALDI-TOF MS)
iii. Immunoassay: Lateral flow immunoassays using specific antibiotics to detect carbapenemase enzyme.

Modified Hodge test: The test is performed by inoculating the plate with lawn culture of Carbapenem susceptible *Escherichia coli* strain. The Ertapenem or Meropenem disc is placed in the centre. The test isolate is streaked away from the disc towards periphery. If the strain is

carbapenemase producing, it will destroy the carbapenem from the disc near its. Site of inoculation enabling the *E.coli* to grow in that region. This gives a clover leaf appearance. This assay is useful for KPC enzymes but it is less sensitive for MBLs.

MHT has limited specificity since extended spectrum beta lactamases (ESBL) or AMP C Cephalosporin's along with porin mutations can result in false positive MHT results. Its specificity is reported to be 91%.

Carba NP test: The test measures in vitro hydrolysis of Imipenem carbapenem in bacterial extract and there is a change in colour of phenol red indicator to yellow due to change in pH. Many variants of this test are available

Carbapenem inactivation method (CIM): when a meropenem disc (10ul) is incubated for 2hrs in water in which 10ul of carbapenemase producing isolate is added, Meropenem gets hydrolysed. This disc is them placed on a lawn culture of sensitive *E.coli* strain and incubated overnight. This will not produce any zone of inhibition. If the test strain does not produce carbapenemase, then there will be zone of inhibition in the lawn culture of sensitive *E.coli.* The CIM test has sensitivity of 91-94% and specificity of 99-100%. This test is later modified by preparing bacterial suspension in tryptic soy broth instead of water and incubating it for 4hrs. This is mCIM test. Due to this modification the sensitivity of the test improved. This mCIM test however cannot distinguish between serine Carbapenemase and Metallo-beta-lactamases. Hence, a further modification is used by addition of EDTA. It is observed that if a strain produces MBL, EDTA will inhibit and MBL production, hence meropenem in the disc is not hydrolysed and will give zone of inhibition in lawn culture seeded with susceptible *E.coli.* Therefore for carbapenemase detection by CIM, mCIM & eCIM are tested in parallel. In this method, along with mCIM test, in second TSB tube20ul of 0.5 M EDTA is added. After 4 hrs incubation the meropenem discs of both tubes are placed on M.H. plates seeded with sensitive *E. coli.* (ATCC 25-25922). If mCIM is positive i.e. there is a zone of inhibition in the plate, then the test strain in the tube is carbapenemase producing 5mm increase in the zone of inhibition in disc of eCIM tube indicates that this carbapenemase is MBL. For *Pseudomonas* and *Acinetobacter* yet another modification using 0.5m Tris-HCL buffer for extraction is found to be useful

Lateral flow immunoassays (LFIA): These are antibody based methods developed to detect one or more epidemiologically important Carbapenemses like NDM IMP, OXA-48 like, KPC & OXA-48 like, KPC, NDM & OXA 48 like an LFIA that targets five important families viz KPC, NDM, VIM, IMP & OXA 48 LIKE carbapenemase has also been evaluated

MALDI TOF MS: This platform is mainly used for identification of microbial genus and species. In MALDI TOF two approaches are being pursued for rapid identification of carbapenemase production. In hydrolysis approach Carbapenem degradation products are detected when bacterial protein extracts are incubated with Carbapenem (Papagiannitsis CC, & et al 2015). In plasmid associated peak approach known carbapenemase being plasmid associated protein peak is detected. MALDI TOF MS can be a cost effective method for Carbapenemase detection, if the instrument is already installed in the laboratory.

Genotypic detection of Carbapenemases:

Carbapenemase genes: With the adult of better molecular diagnostic methods, various genes encoding carbapenemases of all three ambler classes, A, B, & D are detected singleplex & multiplex PCR test are now available for five predominant Carbapenemase genes viz blaKPC genes (KPC), blaIMP, blaVIM & blaNDM (MBLs) , blaOXA-48 gene (OXA-48)

The blaKPC gene is common in unites states, while MBL, especially blaNDM is common in

Indian subcontinent. OXA is seen all over the world, likewise several studies have documented simultaneous detection of more than one gene of carbapenemases. Even two unrelated carbapenemase genes have been detected in earlier studies

CONCLUSION:

Many phenotypic as well as genotypic methods are available for carbapenemase detection. The phenotypic methods have different sensitivities and specificities and may not always be able to all types of Carbapenemases. Some may take longer time due to requirement of overnight incubation. However phenotypic tests are simple, easy to perform and cost effective as compared to genotypic tests. Genotypic tests are more specific and more sensitive however they require better equipped laboratories with trained staff and definitely more expensive as on date.

It also been observed that Carbapenemase production is predominantly seen in MDR, XDR and PDR organisms and indeed poses red problems in choosing the suitable antibiotics. The detection of carbapenemase will certainly help to choose the antibiotics earlier when multidrug resistance pathogens are encountered.

REFERENCES:

Pawar S K, Mohite S T,Shinde R V, Patil S R, Karande G S. Carbapenem – Resistant Enterobacteriaceae: Prevalence and bacteriological profile in a tertiary teaching hospital from rural western India – Indian Journal of Microbiology Research (Ip Innovative Publication Pvt.Ltd.) 2018;5(3):342-347. https://doi.org/10.18231/2391-5478.2018.0072

Sheikh A F, Shahin M, Shokoohizadeh L, Ghanbari F, Solgi H, Shahcheraghi F. Emerge of NDM-1producimg multidrug resistant pseudomonas aeruginosa and co- harboring of carbapenemase genes in South Iran. 2020, Iranian Journal of public health, Vol. 49 (5), p. p 959.

Ambler R P. The structure of beta lactamases. 1980, Philos Trans R Soc Lond B Biol Sci 289:, pp. 321-331.

Gniadek T J, Carroll K C, Simner P J. Carbapenem-resistant non–glucose-fementing Gram negative bacilli: the missing Piece to the puzzle. 2016, Journal of clinical Microbiology, Vol. vol 54, pp. 1700-1710.

Shaker O A, Gomaa H E, Eimasry S A, Halim R M A, Abdelrahman A H, Kamal J S. Evaluation of Combined Use of Temocillin Disk and Mastdisks Inhibitor Combination Set Against Polymerase Chain Reaction for Detection of Carbapenem-Resistant Enterobacteriaceae. 2018, Maced Journal of Medical Science, Vol. Feb 15; 6 (2), pp. 242–247.

Braun S D, Jamil B, Syed M A, Abbasi S A, Waib D, Slickers P, Monecke S, Engelmann I, Ehricht R. Prevalence of carbapenemase-producing organisms at the Kidney Center of Rawalpindi (Pakistan) and evaluation of an advanced molecular microarray-based carbapenemase assay. 2018, Future microbiology, Vol. 13(11), p. p. 314.

Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, Zheng Y, Guo Y, Zhang R, Hu F. Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among Carbapenem-Resistant Enterobacteriaceae isolated from adult and children patients in China. . 2020, Frontiers in Cellular and Infection Microbiology, Vol. vol 10, p. p. 314.

Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for detection of emerging carbapenemases in Enterobacteriaceae. 2012, Journal of

Clinical Microbiology, Vol. vol 50, pp. 477-479.

Carvalhaes C G, Picao R C, Nicoletti A G, Xavier D E, Gales A C. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in Klebsiella pneumoniae: be aware of false positive results. 2010, Journal of Antimicrob Chemother, Vol. vol 65, pp. 249–251.

Tamma P D, Opene B N,Gluck A, Chambers K K, Carroll K C, Simmer P J. Comparison of 11 phenotypic assays for accurate detection of carbapenemase-producing Enteroabacteriaceae. 55: 2017, Journal of Clinical Microbiology, Vol. vol 55, pp. 1046-1055.

Vasco S, Cunningham S A, Kohner P C, Simmer P J, Mandrekar J N, LOlans K, Hayden M K, Patel R. Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. 2013, Journal of clinical microbiology, pp. 3097-3101.

Nordmann P, Poirel L, Dortet L. Rapid Detection of carbapenemase-producing Enterobacteriaceae. 2012, Emerg Infect Dis, Vol. vol 18, pp. 1503-1507.

Pires J, Novais A, Peixe L. Blue-Carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. 2013, Journal Clinical Microbiology , Vol. vol 51, pp. 4281–4283.

Bakour S, Garcia V, Loucif L, Brunel J M, Gharout-Sait A, Touati A, Rolain J M. Rapid identification of carbapenemase-producing Enterobac- teriaceae, Pseudomonas aeruginosa and Acinetobacter baumannii using a modified Carba NP test. 2015, New Microbes New Infection, pp. 89-83.

Sun K, Xu X, Yan J, Zhang L. Evaluation of six phenotypic methods for the detection of carbapenemases in Gram-negative bacteria with characterized resistance mechanisms. 2017, Ann Lab Med, Vol. vol 37, pp. 305-312.

Pierce V M, Simner P J, Lonsway D R, Roe-Carpenter D E, Johnson J K, Brasso W B, Bobenchik A M, Lockett Z C, Charnot-Katsikas A, Ferraro M J, Thomson R B, Jr, Jenkins S G, Limbago B M, Das S. Modified carbapenem inactivation method for phenotypic detection of carbapenem production among enterobacteriacae. 2017, Journal of clinical microbiology, Vol. vol 55.

Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing, 28th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.

Uechi K, Tada T, Shimada K, Kuwahara-Arai K, Arakaki M, Tome T, Nakasone I, Maeda S, Kirikae T, Fujita J. A modified carbapenem inactivation method, CIMTris, for carbapenemase production in Acineto- bacter and Pseudomonas species. 2017, Journal of clinical microbiology, Vol. vol 55.

Dortet L, Jousset A, Sainte-Rose V, Cuzon G, Naas T. Prospective evaluation of the OXA-48 K-SeT assay, an immunochromatographic test for the rapid detection of OXA-48-type carbapenemases. 2016, Journal of Antimicrob Chemotherapy, Vol. vol 71, pp. 1834 –1840.

Notake S, Matsuda M, Tamai K, Yanagisawa H, Hiramatsu K, Kikuchi K. Detection of IMP metallo-beta-lactamase in carbapenem-nonsusceptible Enterobacteriaceae and non-glucose-fermenting Gram-negative

rods by immunochromatography assay. 2013, Journal of Clinical Microbiology.

Glupczynski Y, Evrard S, Ote I, Mertens P, Huang TD, Leclipteux T, Bogaerts P. Evaluation of two new commercial immunochromato- graphic assays for the rapid detection of OXA-48 and KPC carbapen- emases from cultured bacteria. 2016, Journal of Antimicrobial Chemotherapy, Vol. vol 71, pp. 1217-1222.

Saleh A, Gottig S, Hamprecht A G. Multiplex immunochromato- graphic detection of OXA-48, KPC, and NDM carbapenemases: impact of inoculum, antibiotics, and agar.. 2018, Journal of Clinical Microbiology.

Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, Plai- sance M, Oueslati S, Dortet L, Jousset A, Simon S, Naas T, Volland H. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-4. 2018, Journal of antimicrobial chemotherapy, Vol. vol 73, pp. 909-915.

Lau A F, Wang H, Weingarten R A, Drake S K, Suffredini A F, Garfield M K, Chen Y, Gucek M, Youn J H, Stock F, Tso H, DeLeo J, Cimino J J, Frank K M, Dekker J P. A rapid matrix-assisted laser desorption ionization–time of flight mass spectrometry-based method for single-plasmid tracking in an outbreak of carbapenem-resistant Enterobacteriacae. 2014, Journal Clinical of microbiology laboratory, Vol. vol 52, pp. 2804-2812.

Youn J H, Drake S K, Weingarten R A, Frank K M, Dekker J P, Lau A F. Clinical performance of a matrix-assisted laser desorption ionization–time of flight mass spectrometry method for detection of certain blaKPC-containing plasmids. . 2016, Journal Clinical Microbiology.

Haji S H, Aka S T H, Ali F A., Prevalence and characterization of carbapenemase encoding genes in multidrug resistant Gram-negative bacilli. PLoS One. 2021 Nov 1;16(11). https://doi.org/10.1371/journal.pone.0259005

Jalalvand K, Shayanfar N, Shshcheraghi F, Amini E, Mohammadpour M, Babaheidarian P. Evalution of phenotypic and genotypic characteristics of carbapenemases-producing enteribacteriaceae and its prevalence in a referral hospital in Tehran city. 2020, Iranian journal of pathology, Vol. vol 15 , p. 86.

Dandachi I, Sokhn E S, Najem E, Azar E, Daoud Z. Carriage of beta lactamase- producing enterobacteriaceae among nursing home residents in north. 2016, International journal of infectious diseases., Vol. vol 45, pp. 24-31.

Poirel L, Walsh T R, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. 2011, Diagnostics microbiology and infectious diseases, Vol. vol 70 (1), pp. 119-123.

Pranita D Tamma, Patricia J. Simner. Phenotypic detection of Carbapenemase producing Organism from clinical isolates .journal of clinical Microbiology. 2018 oct 25;56(11). https://doi.org/10.1128/JCM.01140-18

Karabay O, Altindis M, Koroglu M, Karatuna O, Aydemir O A, Erdem A F. The carbapenem-resistant Enterobacteriaceae threat is growing: NDM-1 epidemic at a training hospital in Turkey. 2016, Annals of clinical microbiology and antimicrobials, Vol. vol 15, pp. 1-6.

Sadeghi M R, Ghotaslou R, Akhi M T, Asgharzadeh M, Hasani A. Molecular characterization of extended-spectrum β-lactamase, plasmid-mediated AmpC cephalosporinase and carbapenemase genes among Enterobacteriaceae isolates in five medical centres of East and West Azerbaijan, Iran. 2016, Journal of medical microbiology, Vol. vol 65, pp. 1322-1331.

Bourafa N, Chaalal W, Bakour S, Lalaoui R, Boutefnouchet N, Diene S M, Rolain J-M. Molecular characterization of carbapenem-resistant Gram-negative bacilli clinical isolates in Algeria. 2018, Infection and drug resistance, Vol. vol 11, p. 735.

Karuniawati A, Saharman Y.R., Lestari D.C. Detection of carbapenemase encoding genes in Enterobacteriace, Pseudomonas Aeruginosa, and Acinetobacter Baumanii isolated from patients at intensive care unit Cipto Mangunkusmo Hospital in 2011. 2013, Acta Med Ind, pp. 101-106.

Codjoe, F.S. Detection and characterisation of carbapenem-resistant gram-negative Bacilli infections in Ghana. 2016, Sheffield Hallam University.

Okoche Dm Asiimwe B B, Katabazi F A, Kato L, Najjuka C F. Prevalence and characterization of carbapenem-resistant Enterobacteriaceae isolated from Mulago National Referral Hospital, Uganda PLoS One. 2015, Vol. 10(8).

Ambler R P. The structure of Beta Lactamase. 1980, Biological science.

Papagiannitsis C C, Studentova V, Izdebski R, Oikonomou O, Pfeifer Y, Petinaki E, Hrabak J. Matrix-assisted laser desorption ionization- time of flight mass spectrometry meropenem hydrolysis assay with NH4HCO3, a reliable tool for direct detection of carbapenemase activity. 2015.

Tamma PD, Opene BN,Gluck A, Chambers KK, Carroll KC, Simmer PJ . Comparison of 11 phenotypic assays for accurate detection of carbapenemase-producing Enteroabacteriaceae. . Tamma PD, Opene BN,Gluck A, Chambers KK, Carroll KC, Simmer PJ. 2017, Journal of Clinical Microbiology, Vol. vol 55, pp. 1046-1055.