Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies ***NLM ID:*** *101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Indian Citation Index (ICI) Index Copernicus value = 100

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Molecular detection of Carbapenemase genes among Gram-negative bacilli isolates recovered from blood stream infections in a tertiary care hospital

Pramodhini S

Professor, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Puducherry, India

ABSTRACT:

Background: Carbapenem resistance in Gram-negative bacteria, especially when Carbapenemase are involved, is the main contributing factor for MDR and usually the definitive step before pandrug resistance (PDR).The objective of this to study to detect and differentiate Carbapenemase (KPC, NDM, VIM, IMP, and OXA-48) producing organisms (*Enterobacterales, Pseudomonas and Acinetobacter*) from blood stream isolates by Xpert Carba-R assay. **Materials and Method:** A total of 50 carbapenem resistant gram-negative bacterial isolates which included *Enterobacterales, Pseudomonas aeruginosa* and *Acinetobacter* were subjected identification of Carbapenemase-encoding genes such blaKPC, blaNDM, blaVIM, blaOXA-48-type, and blaIMP by Xpert Carba -R assay. **Results:** Study of carbapenem resistance(CR) gene by Xpert Carba-R detected CR gene among 35/50 (70%) isolates and no CR gene detected among 15/50(30%) isolates. Among 35 isolates, where CR gene detected, 15/35 (30%) had both NDM and OXA 48, 15/35 (30%) had only NDM and 5/135(10%) had OXA 48. Among Enterobacterales, in 86.5% of isolates carbapenemase gene detected and 13.5% no gene detected, whereas among non fermenters, 23.1% of isolates carbapenemase gene detected and 76.9 % no CR gene was detected. **Conclusion:** In our study, the most prevalent CR gene was NDM and the coexistence of NDM, with OXA-48 was common among *Enterobacterales.* None of the nonfermenters in our study exhibited OXA-48 gene. Understanding the need for geographically-specific distribution pattern of common resistant mechanisms that codes for carbapenemase production both from community and hospital settings, which help to formulate therapeutic options to combat infections caused by carbapenem-resistant Gram-negative bacteria (GNB).

Key words: Carbapenemases, *Enterobacterales*, NDM, OXA-48, Xpert Carba -R assay

Received: 11 July, 2023 Accepted: 13 August, 2023

Corresponding Author: Pramodhini S, Professor, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Puducherry, India

This article may be cited as: S Pramodhini. Molecular detection of Carbapenemase genes among Gram-negative bacilli isolates recovered from blood stream infections in a tertiary care hospital. J Adv Med Dent Scie Res 2023;11(9):68-73.

INTRODUCTION

Carbapenem resistance in Gram-negative bacteria due to production of enzyme carbapenemases, is the main root cause for Multidrug resistance (MDR), and usually the definitive step before developing pandrug resistance (PDR)[1].

In gram negative pathogens, resistance to carbapenem is either by production of enzyme carbapenemases which is plasmid mediated or by efflux pump and porin loss due to chromosomal mechanism. Carbapenemase producing gram-negative bacteria (GNB) has become a major problem in most of the tertiary care centers worldwide. They are often cause invasive infections associated with high morbidity and mortality rates[2].For initiating prompt and adequate antibiotic therapy as well as infection control, rapid detection of multidrug resistance organisms is very important.

In general, most of the clinical laboratories identifies carbapenemase production either by phenotypic methods such as mCIM, mCIM plus eCIM or CarbaNPor molecular method of detection of carbapenemase-encoding genes such blaKPC, blaNDM, blaVIM, blaOXA-48-type, and blaIMP. However, it is not possible to detect all carbapenemases and other resistance mechanisms that may results in carbapenem resistance[3].

The objective of this to study to detect and differentiate Carbapenemase (KPC, NDM, VIM, IMP, and OXA-48) producing organisms (*Enterobacterales,*

Pseudomonas and Acinetobacter) from blood stream isolates by Xpert Carba-R assay.

MATERIALS AND METHODS

A total of 50carbapenem resistant gram-negative bacteria isolated from blood stream infections for a period of ten months from October 2022 to May 2023 were included in the study.Identification and MIC determination were performed using VITEK®2 compact system (bioMérieux). Organisms that were resistant to imipenem and meropenem wereinterpreted according to the Clinical Laboratory Standards Institute, guidelines (CLSI-M1OO-S33)[4]Among gram negative bacterial pathogens,*Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* that were found resistant to carbapenems were further subjected to detection of carbapenemase-encoding genes such blaKPC, blaNDM, blaVIM, blaOXA-48-type, and blaIMP by Xpert Carba -R assay.

The study was approved by the Institutional Human Ethics Committee as per Indian Council of MedicalResearch guidelines 2017 and was performedaccording to the WMA Declaration of Helsinkiethical principles for research on humans.

STATISTICAL ANALYSIS

All data will be entered into MS Excel 2010 and analyzed. Qualitative variables represented by frequency and percentage.

RESULTS

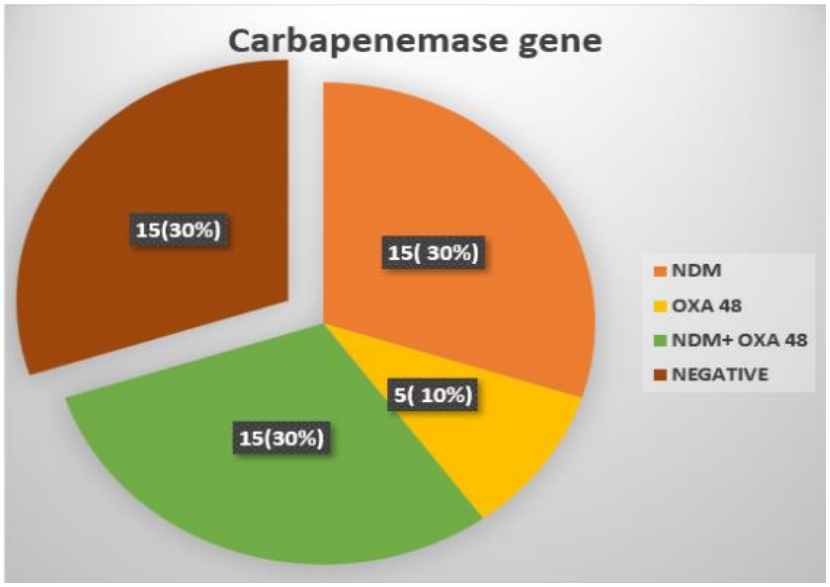
A total of 50-gram negative bacterial isolates were studied, which included37 Enterobacterales and 13 Nonfermenting gram negative bacilli (NFGNB). Among 37 Enterobacterales, 29 were *Klebsiella pneumoniae*, 3 *Escherichia coli*, 2 *Serratia marscescens* and 1 each of *Pantoea spp,Enterobacter cloacae* and *Enterobacter aerogenes*.Of 13 NFGNB, included 10 *Acinetobacter baumanii* and 3 *Pseudomonas aeruginosa*(Table 1).

Table 1: Distribution pattern of Carbapenem resistant gene in CR-Gram negative organism (n=50)

ISOLATE	NDM	OXA 48	NDM+OXA 48	No Gene detected	TOTAL
Enterobacterales (37)					
Klebsiella pneumoniae	7	5	14	3	29
Pantoea spp	1			0	1
Serratia marscescens	2			0	2
Enterobacter cloacae			1	0	1
Enterobacter aerogenes				1	1
Escherichia coli	2			1	3
Non fermenting GNB (13)					
Acinetobacter baumanii	2			8	10
Pseudomonas aeruginosa	1			2	3
TOTAL	**15(30%)**	**5(10%)**	**15(30%)**	**15(30%)**	**50**

Study of carbapenem resistance(CR) gene by Xpert Carba-R detectedCR gene among 35/50(70%) isolates and no CR gene detected among 15/50(30%) isolates. Among 35 isolates of CR gene detected, 15/35 (30%)had both NDM and OXA 48, 15/35 (30%) had only NDM and 5/135(10%) had OXA 48 (Figure 1).

Figure 1: Pattern of distribution of carbapenemase genes in blood stream isolates



Among Enterobacterales, in 86.5% of isolates carbapenemase gene detected and 13.5% no CR gene detected,

whereas among non-fermenters, 23.1% of isolates had CR gene and 76.9 % no CR gene was detected (Figure 2) and the graph showing various CRgene pattern and no CR gene pattern are given in Figure 3.

Figure 2: Percentage of carbapenemase gene detected among Enterobacterales and Nonfermenting Gram negative bacilli(NFGNB)

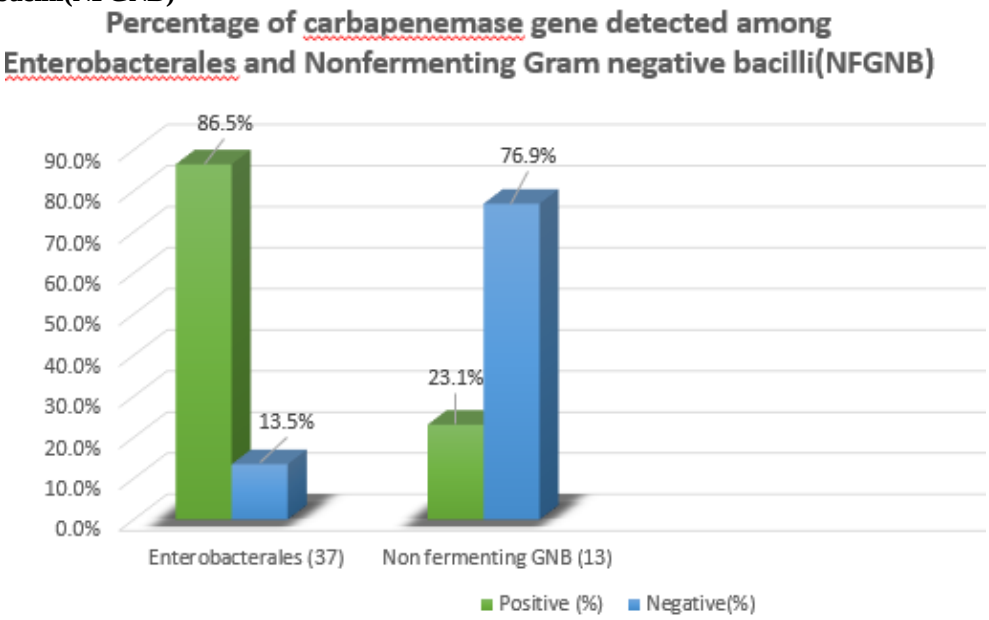
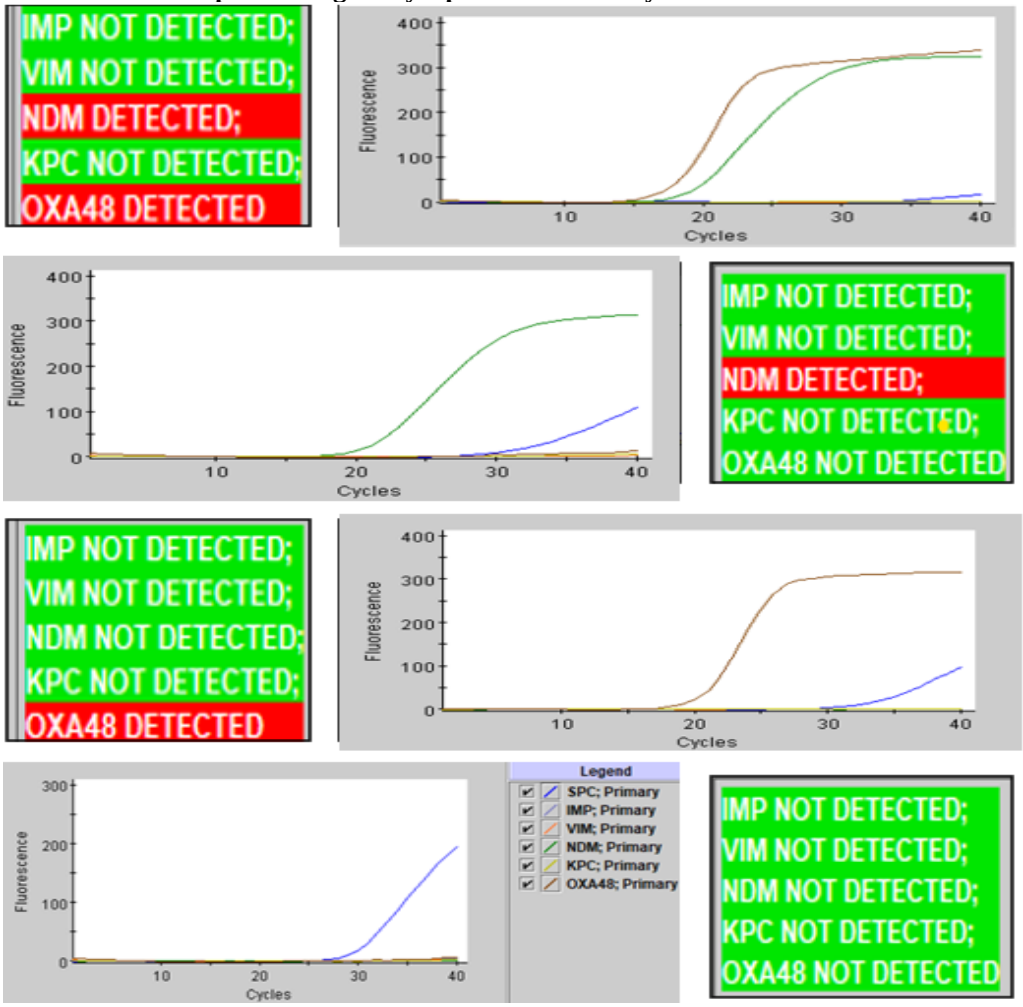
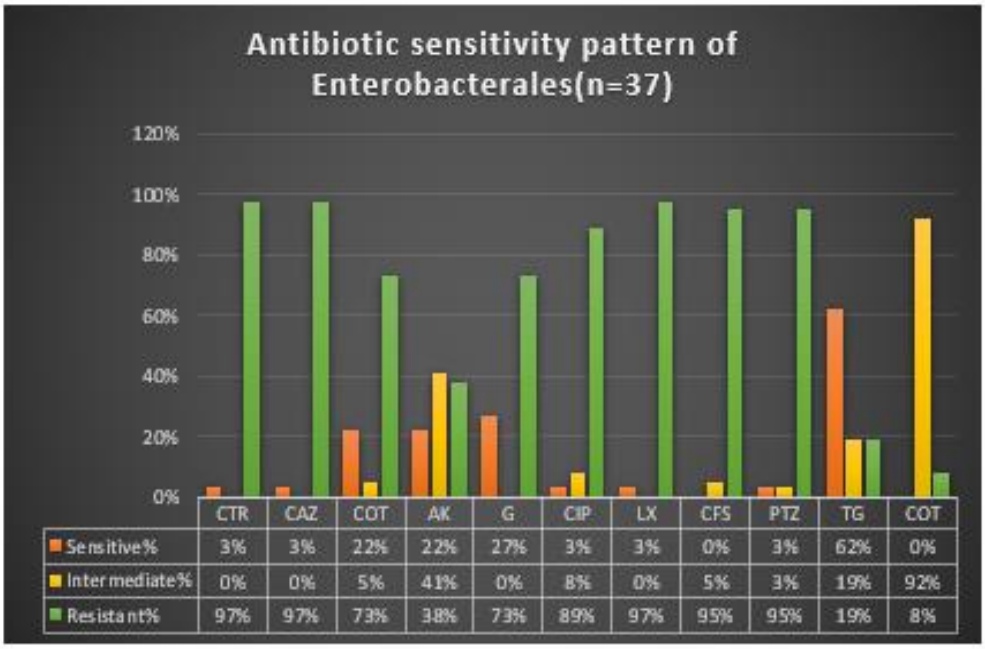


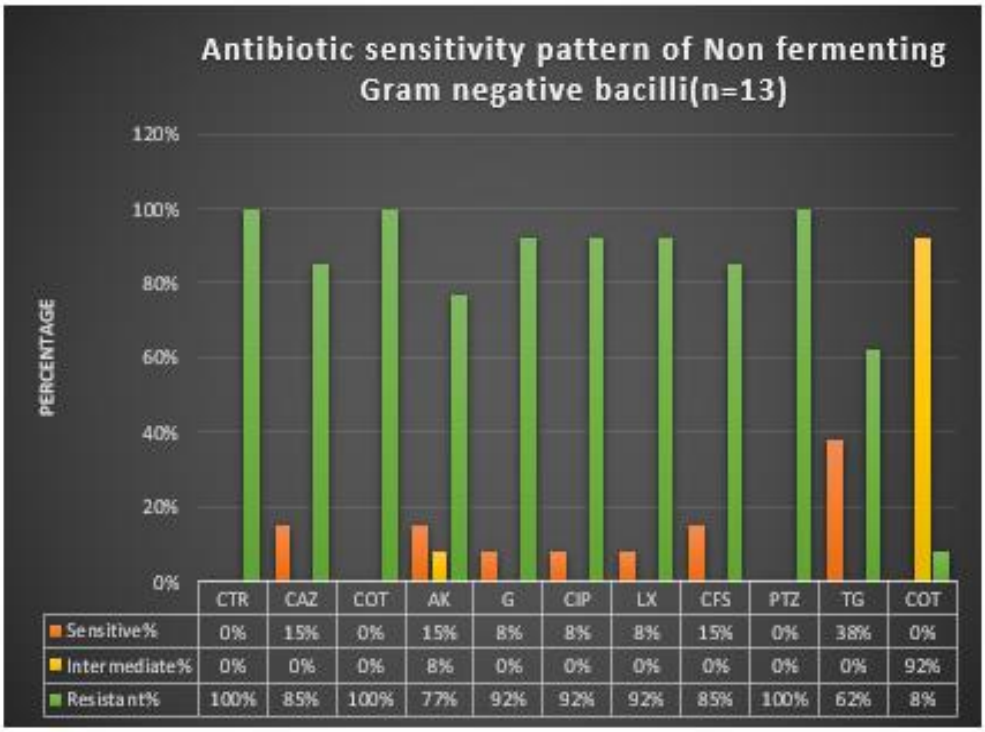
Figure 3: Pattern of Carbapenemase gene by Xpert CarbaR assay



Antimicrobial sensitivity pattern of carbapenem resistant isolates showed more than 95% resistance to third generation cephalosporins, fluoroquinolones, Cefperazone sulbactum and Piperacillin tazobactum. Sensitivity pattern for aminoglycosides among enterobacterales showed 38% and 73% resistant to amikacin and gentamicin respectively, and nonfermentors showed 77% and 92% resistant to amikacin and gentamicin respectively. Sensitivity to tigecycline was 62% and 38% for enterobacterales and nonfermentors respectively, whereas 92% intermediate sensitivityfor Colistin was noticed among *Enterobacterales* and nonfermentors (Figure 4).

Figure 4: Antibiotic susceptibility pattern of Carbapenem resistant Enterobacterales and Non fermenting Gram-negative bacilli (n=50)





CTR-Ceftriaxone, CAZ-Ceftazidime, COT-Cotrimoxazole, AK-Amikacin, G-Gentamicin, CIP-Ciprofloxacin, LX-Levofloxacin, CFS-Cefperazone sulbactum, PTZ-Piperacillin Tazobactum, TG-Tigecycline, Co-Colistin

DISCUSSION

Carbapenem resistance in gram negative bacteria can be by decreased permeability, overexpression of efflux pump, mutation and transformation in antibiotic target structures, and modification of antibiotics by the hydrolysis of the molecule.Carbapenemase production is the most common mechanism of resistance to carbapenems in *Enterobacterales*. Carbapenemases are found in three classes of β-lactamases such as class A or D serine β-lactamases and class B metallo-β-lactamases (MBLs). The most common carbapenemase genes are the *Klebsiella pneumoniae* carbapenemase gene (blaKPC, class A), the New Delhi metallo-β-lactamase gene (blaNDM, class B), the Verona integron-encoded metallo-β-lactamase gene (blaVIM, class B), the imipenemase metallo-β-lactamase gene (blaIMP, class B), and the oxacillinase-48 gene (blaOXA-48, class D). Because most of these genes encoding carbapenemase are plasmid mediated, carbapenem resistance can disseminate rapidly throughout different regions.

In *Pseudomonas aeruginosa*, nonenzymatic carbapenem resistance mechanisms is due to overexpression of efflux pumps and loss of expression of porin-encoding genes, mutations in chromosomally encoded porin genes (such as OprD). Whereasin *Acinetobacter* isolates, non-carbapenemase specific mechanisms such as mixture of porin loss in addition with specific efflux pump systems have been reported.Other Carbapenem resistance mechanisms rarely observed in *Escherichia coli, P. aeruginosa* and *A. baumannii*can also be due to mutations or modifications that can alter the production level or the binding affinity of penicillin-binding proteins[5].

In our study, NDM(30%) and the co-production of NDM, with OXA-48 (30%) were the most frequent gene detected, followed by absence of any CR gene in (30%) and OXA-48 production in 10% (5/35).

Study by Nayak *et al*[6],had reported NDM was the most frequent gene (34.6%), followed by the co-production of NDM and OXA-48 (25%), absence of any CR gene (25%) and OXA-48-like alleles production (15.3%), comparable to our study.Vamsi *et al*, in their study identified NDM (47.3%) most common genes, followed by the co-existence of genes in combination of NDM, with VIM (39.6%), VIM and OXA-48 (4.3%), and OXA-48 (1.5%)[7].New Delhi metallo-β-lactamase (47.3%) was the pre-dominant gene in the study reported by Naim *et al,*consistent with our study[8].

The coexistence of these carbapenemase genes is a therapeutic challenge to clinicians, due to restricted treatment options and the potential for global spread by horizontal transfer. As noticed, some isolates were found to contain two unrelated carbapenemase genes (bla OXA-48 and bla NDM) as previously observed in other studies which had co-existence of NDM and OXA-48 genes 24.44%[9] and 20%[10]among enterobacterales, consistent with the findings of our study.In a study by Grag *et al,* identified the NDM and OXA-48 co-existence pre-dominantly in *E.coli,* whereas we have identified the NDM and OXA-48 co-existence more common in *Klebsiella spp*. None of the nonfermenters in our study exhibited OXA-48 gene. Further in our study, CR genes blaKPC, blaVIM, and blaIMP were not detected, which was comparable with the observations made by Garg *et al*[10].

In our study, 30% of carbapenems resistant isolates had no CR gene detected by Xpert CarbaR, which can be attributed to other resistance mechanisms other than carbapenemase such as decreased permeability, overexpression of efflux pump, mutation and transformation in antibiotic target structures.

The antimicrobial susceptibility profiles, among carbapenemase-producing isolates had demonstrated increased resistance level 3rd cephalosporins,quinolones and aminoglycosides andmost of the isolates showed increased susceptibility to colistin and tigecycline in comparison to other study [7].

Understanding the need for geographically-specific distribution pattern of common resistant mechanisms that codes for carbapenemase production both from community and hospital settings, which help to formulate therapeutic options to combat infections caused by carbapenem-resistant Gram-negative bacteria (GNB).

CONCLUSION

In our study, the most prevalent CR gene wasbla NDM and the coexistence of bla OXA-48 and bla NDM was common among *Enterobacterales.*None of the non fermenters in our study exhibited bla OXA-48 gene. Emergence of carbapenemase-producing Gram-negative pathogens in blood stream infections is an alarming situation in healthcare-related infections. Early detection and isolation of patients infected or colonized with strains that carbapenemase genesis essential in treating patients, as well as implementing appropriate infection control measures to curb the rapid spread of these strains.

ACKNOWLEDGMENTS

The author would like to acknowledge the Sri Balaji Vidyapeeth (Deemed to be University) for intramural funding and Department of Microbiology, MGMCRI for their supportin carrying out the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis. 2016 Feb;3(1):15-21. doi: 10.1177/2049936115621709. PMID: 26862399; PMCID: PMC4735501
2. Kumar M, Tandel K, Shergill SPS, Bhalla GS, Mahajan P, Swarnim V, Sahai K, Gupta RM. Rapid detection of

carbapenem resistance among gram-negative organisms directly from positive blood culture bottles. Med J Armed Forces India. 2023 May-Jun;79(3):267-274. doi: 10.1016/j.mjafi.2021.03.026. Epub 2021 Jun 25. PMID: 37193528; PMCID: PMC10182285.

3. Codjoe FS, Donkor ES. Carbapenem Resistance: A Review. Med Sci (Basel). 2017 Dec 21;6(1):1. doi: 10.3390/medsci6010001. PMID: 29267233; PMCID: PMC5872158.
4. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 33rd informational supplement. CLSI Document M100-S33 2023 Wayne, PA CLSI
5. Patrice Nordmann, Laurent Poirel, Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria, Clinical Infectious Diseases, Volume 69, Issue Supplement_7, 1 December 2019, Pages S521–S528, https://doi.org/10.1093/cid/ciz824
6. Nayak G, Behera B, Mahapatra A, Tripathy S, Biswal J. Molecular Detection of Carbapenemase Enzymes Directly from Positive Blood Cultures Using Xpert Carba-R. J Lab Physicians. 2022 Apr 19;14(3):365-368. doi: 10.1055/s-0042-1744238. PMID: 36119431; PMCID: PMC9473928.
7. Vamsi SK, Moorthy RS, Hemiliamma MN, Chandra Reddy RB, Chanderakant DJ, Sirikonda S. Phenotypic and genotypic detection of carbapenemase production among gram negative bacteria isolated from hospital acquired infections. Saudi Med J. 2022 Mar;43(3):236-243. doi: 10.15537/smj.2022.43.3.20210809. PMID: 35256490; PMCID: PMC9280532.
8. Naim H, Rizvi M, Gupta R, Azam M, Taneja N, Shukla I, et al.. Drug resistance and molecular epidemiology of carbapenem resistant Gram-negative bacilli isolates. J Glob Infect Dis 2018; 10: 133–139..
9. Jalalvand K, Shayanfar N, Shahcheraghi F, Amini E, Mohammadpour M, Babaheidarian P. Evaluation of Phenotypic and Genotypic Characteristics of Carbapnemases-producing Enterobacteriaceae and Its Prevalence in a Referral Hospital in Tehran City. Iran J Pathol. 2020 Spring;15(2):86-95. doi: 10.30699/ijp.2020.111181.2188. Epub 2020 Feb 28. PMID: 32215024; PMCID: PMC7081758.
10. Garg A, Garg J, Kumar S, Bhattacharya A, Agarwal S, Upadhyay GC.. Molecular epidemiology and therapeutic options of carbapenem-resistant Gram-negative bacteria. Indian J Med Res 2019; 149: 285–289