Molecular Detection of Carbapenemase Enzymes Directly from Positive Blood Cultures Using Xpert Carba-R

Gayatree Nayak[1] Bijayini Behera[1] Ashoka Mahapatra[1] Swagata Tripathy[2] Jyoti Biswal[3]

[1] Department of Microbiology, All India Institute of Medical Sciences, Bhubaneswar, Orissa, India
[2] Department of Anaesthesiology & Critical Care Medicine, All India Institute of Medical Sciences, Bhubaneswar, Orissa, India
[3] Department of Infection Control Nursing, All India Institute of Medical Sciences, Bhubaneswar, Orissa, India

Address for correspondence Bijayini Behera, MD, Department of Microbiology, All India Institute of Medical Sciences (AIIMS), Bhubaneswar, Odisha, 751019, India (e-mail: drbinny2004@gmail.com).

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Abstract

Objective The performance of Xpert Carba-R assay for the direct identification of carbapenemases directly from positive blood culture vials was evaluated.

Materials and Methods In total, 176 positively flagged blood culture vials, yielding carbapenem-resistant GNB (CR-GNB), were enrolled for the detection and differentiation of blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP using Xpert Carba-R.

Results *Klebsiella pneumoniae* (76/176, 43.1%), *Acinetobacter baumannii* complex (67/176, 38%), and *Escherichia coli* (29/176,16.4%) were the predominant isolates. Overall, NDM production was the commonest (61/176, 34.6%), followed by the co-production of NDM + OXA-48 and the absence of any CR gene (44/176, 25%), followed by OXA-48 (27/176, 15.3%). In CR *K. pneumoniae*, the co-production of NDM + OXA-48 was most frequent (34/76, 44.7%), whereas in the *A. baumannii complex*, no CR gene was detected in the majority of isolates (38/67, 56.7%). *bla NDM* was the commonest gene in *E. coli* (18/29, 62%) and *A. baumannii* complex (26/67, 38.8%).

Conclusion Xpert Carba-R can identify the molecular mechanism of CR within hours after a blood culture turns positive and, thus, has the potential for optimization of antimicrobial therapy, choosing appropriate novel β-lactam combination agents, as well as infection control interventions.

Keywords
- blood culture
- carbapenemase
- Xpert carba-R

Introduction

Carbapenemase-producing gram-negative bacteria (CP-GNB) are emerging causes of bloodstream infections (BSI) and are associated with significant mortality.[1] Carbapenemase enzymes are organized into three classes: Class A β-lactamases such as *Klebsiella pneumoniae* carbapenemase (KPC), Class B metallo-β-lactamases (MBL) including imipenemase (IMP), Verona-Integron encoded MBL (VIM), New Delhi-metallo β-lactamases (NDM), and the class D carbapenem hydrolyzing oxacillinases.[2] Considerable geographical variation exists in the prevalence and distribution of

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carbapenemases. Early identification of type of carbapenemase is crucial for the optimization of antimicrobial therapy. The Gene Xpert Carba-R assay (Cepheid, Sunnyvale, CA 94.089, United States) is an FDA-approved CE-IVD marked assay for the simultaneous detection and differentiation of five major carbapenemases, namely, blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP from rectal swabs and pure colonies.[3] There are a few published studies on the validation and applicability of Gene Xpert Carba-R directly from positive blood culture broths.[4,5] In the present study, we evaluated the performance of the Xpert Carba-R assay for the direct detection and identification of carbapenemases from positive blood culture broths, yielding carbapenem-resistant GNB (CR-GNB). The study was approved by the Institute Ethics Committee (IEC approval no. and date: T/IM-F/19–20/01, June 5, 2020).

Materials and Methods

Blood culture bottles from admitted patients of various intensive care units (ICUs), and inpatient units were incubated in the automated blood culture system (BD BACTEC Plus Aerobic medium; Becton Dickinson and Company, Sparks, MD 21152, United States). Subcultures from positively flagged blood culture vials were performed on 5% sheep blood agar (SBA) and MacConkey agar. Bacterial identification and susceptibility testing were performed using the VITEK 2 compact system (BioMerieux; Marcy l'Etoile, France). Carbapenem resistance was defined as resistance to any one of the carbapenem antibiotics (Ertapenem, Meropenem, Imipenem, Doripenem). All consecutive, non-duplicate blood culture vials, yielding pure growth of CR-GNB were enrolled in the present study. Positively flagged blood culture vials yielding polymicrobial growth on culture were excluded. As we intended to perform the Xpert Carba-R assay after ascertaining organism identification and carbapenem susceptibility, positive blood culture vials were stored in the refrigerator for an average of 48 hours before performing the test. Ten blood culture vials yielding carbapenem-susceptible Gram-negative bacilli were included for initial validation and none of them yielded any CR gene. The Xpert CarbaR cartridges and sample reagent vials were allowed to reach room temperature before the assay. Initially, we were adding 100 µL of positive blood culture broth into the sample reagent as per the method by Jauréguy et al.[6] Out of 15 samples tested following this method, 4 samples gave error code 2008, that is, syringe pressure reading of 100.1, PSI exceeded the protocol limit of 100.0 PSI. Later, 40 µL of sample from the positive blood culture broth were directly mixed with sample reagent buffer, and the Xpert Carba-R cartridge was launched with 1.7 mL of this mix as per the method by Cointe et al[4] and we obtained no further errors related to the syringe pressure reading.

Results

The study included 176 consecutive, non-duplicate positively flagged blood culture vials, yielding carbapenem-resistant GNB from October 2020 to August 2021. In total, 118 were obtained from various ICUs and 58 were obtained from various inpatient wards. *Klebsiella pneumoniae* (76/176, 43.1%), *Acinetobacter baumannii complex* (67/176, 38%), and *Escherichia coli* (29/176, 16.4%) were the predominant isolates. From the positive blood culture broths yielding CR-GNB, NDM was the most frequent gene detected (61/176, 34.6%), followed by the co-production of NDM + OXA-48-like alleles (44/176, 25%), absence of any CR gene (44/176, 25%) and OXA-48-like alleles production (27/176, 15.3%). In CR *Klebsiella pneumoniae*, the co-production of bla NDM+ bla OXA-48-like allelles was the most common mechanism (34/76, 44.7%), followed by the production of bla OXA-48 alone (23/76, 30.2%). In the *A. baumannii complex*, no CR gene was detected in the majority of isolates (38/67,56.7%), and *bla NDM* was the most common detected CR gene (26/67, 38.8%). In *Escherichia coli*, *bla NDM* was the most common detected CR gene (18/29, 62%). The distribution of CR genes in various CR-GNB is depicted in ►**Table 1** and the graph of Xpert Carba R is depicted in ►**Fig. 1**. We also performed amplification both from the broth and pure colonies using a panel of previously published primers with single PCRs for each gene in one isolate each from *A. baumannii complex*, *K. pneumoniae*, and *E. coli*, respectively, having NDM, NDM + OXA-48, and OXA-48 alone.[7] There was absolute concordance between the results of Xpert Carba-R from broth, colonies, and conventional PCR. Overall, high mortality (87/176, 49.4%) was observed in patients with CR-GNB infection. Genus-wise or CR gene-wise stratification of mortality did not yield any significant difference.

Discussion

A few studies have described the off-label validation of Xpert Carba-R directly in positive blood culture broth fluids.

Table 1 The distribution of carbapenem-resistant genes in various CR-GNB

	Escherichia coli	*Klebsiella pneumoniae*	*Acinetobacter baumannii complex*	*Enterobacter cloacae*	*Pseudomonas aeruginosa*	Total
NDM	18	16	26	0	1	61
OXA-48 like allele	2	23	1	1	0	27
NDM + OXA-48	7	34	2	1	0	44
No genes detected	2	3	38	1	0	44
Total	29	76	67	3	1	176

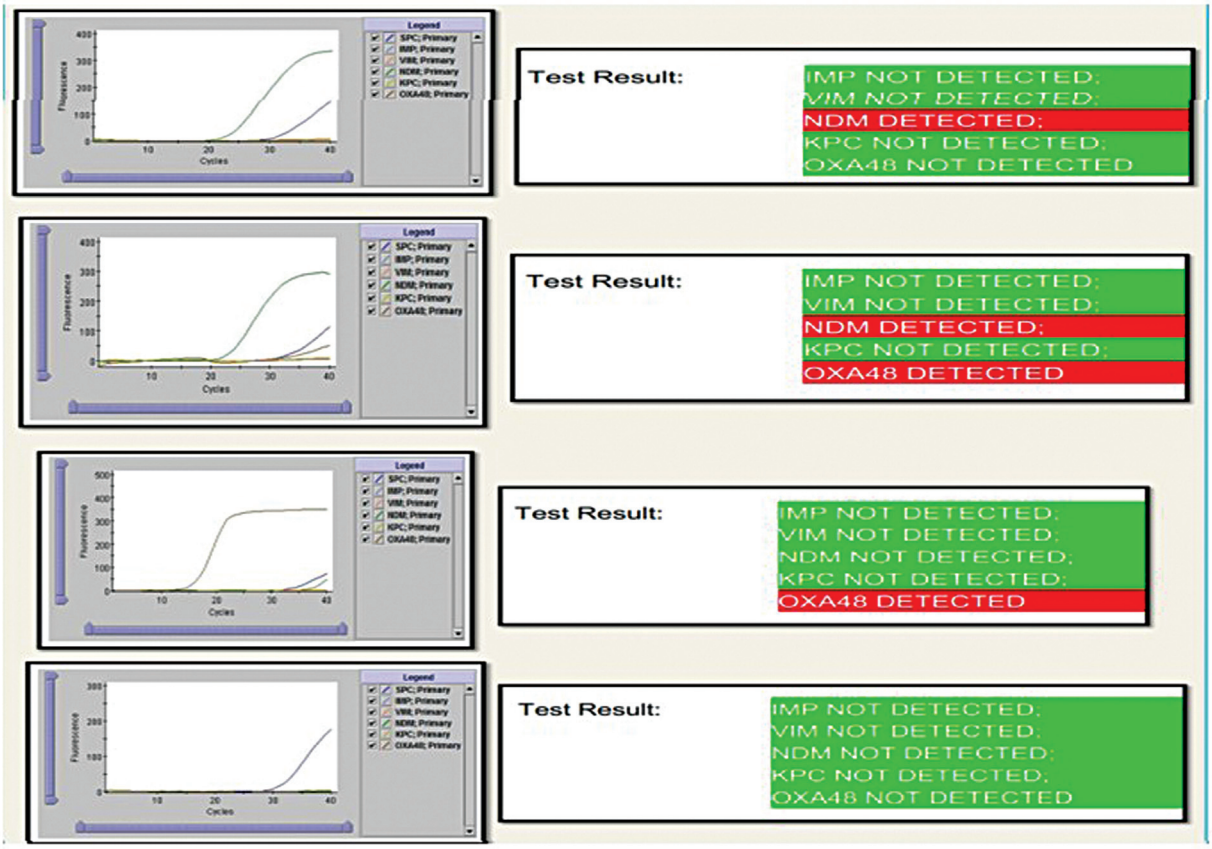

Fig. 1 CABA R graphs.

Cointe et al had evaluated Xpert Carba R in 53 well-characterized isolates and 20 positive blood culture vials, having GNB on Gram stain.[4] A 100% sensitivity and specificity were observed, regardless of the type of carbapenemase and the time of incubation.[4] In an Indian study, evaluating the Xpert Carba-R assay on flagged blood culture samples in ICU patients with Gram-negative bacteremia, OXA-48-like (29/58–50%) was the most frequent gene, followed by NDM (19/58–32.7%), and OXA-48 and NDM co-expression (9/58–15.51%).[5] *K. pneumoniae* was the most common isolate and OXA-48 production was the most common CR gene detected in *K. pneumoniae* similar to our study.[5] In India, CR is predominantly due to the production of NDM and OXA-48-like and KPC is rare.[8] The change in the trend from NDM to OXA-48-like group of enzymes, particularly in *K. pneumoniae* is a notable finding of our study and matches with other studies from Southern and Northern India.[9–11] No CR gene was detected in the majority of *A. baumannii* complex isolates (38/67, 56.7%). This could be either due to the production of oxacillinase enzymes other than OXA-48, for example, OXA-23, OXA-24/40-like, OXA-58, OXA-143-like, OXA-253, and so on,[12] which are not included in the Xpert Carba-R assay or mechanisms such as the overexpression of efflux pumps or porin loss. The approval of novel β-lactam combination agents has made the distinction of carbapenemase enzymes crucial for therapeutic decision, for example, ceftazidime–avibactam for KPC and OXA-48 and aztreonam–avibactam for MBL, etc,[13] and Xpert Carba-R has the potential to facilitate the process. We would like to highlight the limitations of our study. First, it was a single-center study performed on limited number of isolates, and validation of the Xpert Carba-R assay with well-characterized isolates was not attempted on a substantial number of isolates.

Conclusion

Xpert Carba-R can identify the molecular mechanism of CR within hours after a blood culture turns positive and thus has the potential for the optimization of antimicrobial therapy, choosing appropriate novel β-lactam combination agents as well as infection control interventions. As we performed the Xpert Carba-R assay, after the ascertainment of carbapenem resistance using VITEK-2, the effect of early communication of molecular mechanism of CR on antimicrobial escalation, de-escalation, could not be evaluated. The impact of Xpert Carba-R on choosing novel antimicrobials and patient outcomes must be evaluated to integrate into routine clinical practice as well as the spectrum of oxacillinases of Xpert Carba-R should be broadened for individual country-specific uses.

Authors' Contribution

Dr. Gayatree Nayak contributed to acquisition of data, or analysis and interpretation of data, and final approval of the version to be published. Dr. Bijayini Behera contributed to conception and design, acquisition of data, or

analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published. Dr. Ashoka Mahapatra contributed to acquisition of data, or analysis and interpretation of data, and final approval of the version to be published. Dr. Swagata Tripathy contributed to conception and design, acquisition of data, and final approval of the version to be published. Mrs. Jyoti Biswal contributed to acquisition of data, and final approval of the version to be published.

Conflict of Interest
None declared.

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