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Short Communication

Microbiology Section

Rapid Screening for Carbapenem Resistant Organisms: Current Results and Future Approaches

SHALINI ANANDAN[1], SUNGANYA DAMODARAN[2], RADHA GOPI[3], YAMUNA DEVI BAKTHAVATCHALAM[4], BALAJI VEERARAGHAVAN[5]

ABSTRACT

Carbapenem producing *Enterobacteriaceae* (CPE) is a major public health threat. A total of 120 carbapenem resistant *E.coli* (n=32) and *K.pneumoniae* (n=88) from blood stream infections were screened for the presence of carbapenem resistant genes KPC, NDM, IMP, VIM, and OXA-48 like using both conventional multiplex PCR and Xpert® Carba-R test. Additionally 26 faeces samples were directly screened with Xpert® Carba-R test. Of the tested isolates, 40% (n=48) of NDM and 39.2% (n=47/) of OXA-48-like were identified. Co-production of OXA-48 and NDM was seen in 15 (12.5%) isolates. In Xpert® Carba-R test, only NDM was identified in 55% (n=66) of tested isolates. Of the tested faeces samples, 12 were identified as carbapenemase producers: nine with NDM, two with the co-production of NDM and VIM and in *Klebsiella* spp (n=1), NDM and KPC co-production was seen. However, Xpert® Carba-R test fails to detect OXA-48 like as compared with multiplex PCR. The sensitivity, specificity, PPV, NPV of Xpert® Carba-R test was 100%, 77%, 96% and 100% respectively. Incorporation of OXA-48 like specific sequence in the panel of Xpert® Carba-R test may improve its sensitivity and maximize the coverage of assay.

Keywords: Carbapenem producing *Enterobacteriaceae*, KPC, NDM, OXA-48, Xpert® Carba-R, VIM

The emergence and global spread of carbapenem resistant *Enterobacteriaceae* (CRE) is of great concern in health-care settings across the world. Accurate detection of infected patients or patients carrying carbapenem resistant *Enterobacteriaceae*, can significantly influence the management. Rapid identification of CRE with short turnaround time helps in infection control. Xpert® Carba-R is based on real time PCR gives results in less than an hour, having an advantage of rapid implementation of epidemiological measures to control the spread.

The objective of the study was to evaluate the performance of the Xpert® Carba-R using clinical isolates and faeces specimens directly. Xpert® Carba-R is performed on Gene Xpert system, fully automated and integrated for sample preparation, nucleic acid extraction, amplification and detection of targets using real time PCR assays. It involves the use of single-use disposable cartridge that holds PCR reagents and hosts the PCR processes. It is a qualitative *in-vitro* test for rapid detection and differentiation of bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, and bla_{OXA-48} like proprietary sequence. The conventional multiplex PCR is considered as a gold standard method with the turnaround time (TAT) of < 5 hours. While comparing with PCR, Xpert® Carba-R test is simple to perform with the minimal hands-on time of 15 minutes and a short TAT of < 1 hour. The cost per cartridge was $ 54.6 versus $ 116.1 for the culture and in-house PCR (Cepheid, Sunnyvale, CA).

Totally, 120 clinical isolates of ccarbapenem resistant Enterobacteriaceae; *E. coli* (n=32) and *K. pneumoniae* (n=88) collected between January and December 2013 from bloodstream infections were included in the study. Resistance to imipenem and meropenem was detected using CLSI (M100-S24) recommended disk diffusion testing procedure and zone of inhibition interpreted according to the same guidelines. In addition, 26 faeces specimens were directly tested for the presence of carbapenemase genes using Xpert Carba-R test. All the blood stream culture isolates were concurrently, investigated for the five clinically relevant carbapenem resistant coding genes KPC, NDM, IMP, VIM and OXA-48 like [1-3] using conventional multiplex PCR similar to Xpert® Carba-R test.

Of the 120 isolated *Enterobacteriaceae* from blood cultures, the organisms were identified as *E. coli* (n=32) and *K. pneumoniae* (n=88). Conventional PCR identified 40% (n=48) of NDM, followed by 39.2% (n=47) of OXA-48-like. Of the 48 (40%) NDM producing isolates: 62.5% (n=30) and 37.5% (n=18) were *E. coli* and *K. pneumoniae* respectively. In addition, 39.2% (n=47) of OXA-48 like in 17% (n=8) of *E. coli* and 83% (n=39) of *K. pneumoniae*. Fifteen (12.5%) carbapenem resistant isolates were found to be co-producers of OXA-48 and NDM [Table/Fig-1]. Notably, all of the tested isolates were negative for IMP, VIM and KPC genes and 10 (8.3%) isolate were negative for all of the five tested genes. In the Xpert® Carba-R, interestingly NDM was identified in over half of the isolates tested (n=66). Surprisingly 3 NDM genes were additionally identified in Xpert® Carba-R test. However Xpert® Carba-R test failed to pick up OXA-48 like. The identified OXA-48- like variant was found to be OXA-181 by sequencing.

Among the 120 blood isolates subjected to Xpert® Carba-R test, 55% (n=66) showed NDM:71.2% (n=47) in *K.pneumoniae* and 28.7% (n=19) in *E.coli*. Notably, 3 NDM genes were additionally identified in Xpert® Carba-R test which were found to be negative by the conventional multiplex PCR [Table/Fig-1]. Fifty four (45%) of the tested isolates were found to be negative for all the targets tested in Xpert® Carba-R. Randomly, 25 isolates with NDM (n=5) and OXA-48-like (n=20) were selected for sequencing and enzyme variant identification. NDM-1 and OXA-181 were the identified enzyme variants of all the sequenced genes showing 100% similarity with reference sequence deposited in NCBI website (www.ncbi.nlm.nih.gov).

CRO GENES	Conventional multiplex PCR (n=120)	Xpert® Carba-R assay (n=120)
OXA-48 like	47 (39.2%) *E.coli* n=8 (17%), *K. pneumoniae* n=39 (83%)	0
NDM	48 (40%) *E.coli* n=18 (37.5%), *K. pneumoniae* n=30 (62.5%)	66 (55%) *E.coli* n=19 (28.7%) *K.pneumoniae* n=47 (71.2%)
OXA-48 like & NDM	15 (12.5%) *E.coli* n=1 (6.67%), *K.pneumoniae* n=14 (93%)	0

[Table/Fig-1]: Comparison Xpert® Carba-R test with conventional multiplex PCR.

The sensivity, specificity, positive predictive value PPV and negative predictive value (NPV) of Xpert® Carba-R test was 100%, 77%,

96% and 100% respectively [Table/Fig-2]. The Xpert® Carba-R test, missed to detect OXA-48 like because there are 10 known variants of OXA-48, only four variants of targets (blaOXA-48, blaOXA-162, blaOXA-163 and blaOXA-204) were included in Xpert® Carba-R test panel. The remaining six variant needs to be included to improvise the kit. Unfortunately, OXA-181 is the missed target which accounts for the reduced specificity. *Since OXA-181 specific primer sequence was missed in Xpert® Carba-R kit, statistical indices were not calculated for OXA-48 like. Inclusion of OXA-48 variants specific target sequence may improve its specificity and sensitivity.

	Xpert® Carba-R
Sensitivity	100%
Specificity	77%
Positive predictive value (PPV)	96%
Negative predictive value (NPV)	100%

[Table/Fig-2]: Sensitivity, specificity, PPV and NPV of Xpert® Carba-R assay (*other than OXA-181)

A total of 12 carbapenemase producers were identified directly from faeces specimen. The organisms identified were *E. coli* (n=5), *Klebsiella* pneumoniae (n=4), *Citrobacter diversus* (n=1) and NFGNB (n=2). This included NDM in the majority (n=9), two (NFGNB) with co-production of NDM and VIM. In one of the *Klebsiella* spp isolates, co-production of NDM and KPC was seen.

Emerging carbapenem resistance with a wide array of enzyme variants in each of the classes A, B metallo-β-lactamases and class D carbapenemase with various hydrolytic properties complicates the laboratory detection [4]. In most of the laboratory, detection of carbapenemase depends on CLSI recommended modified Hodge test which requires 24 hours of incubation for the interpretation. Recently, CLSI (M100-25) recognised the use of carba NP calorimetric test for rapid detection of carbapenemases. Recently a commercial RAPIDEC® CARBA NP test (Biomerieux, France) works on the principle of carba NP is available with excellent sensitivity (97.8%), specificity (97.8%) and TAT of 2 hrs for better patient management. However, Xpert carba R rapidly detects and differentiate class A, B and class D carbapenemase which is important for epidemiological investigation and infection control measure be instituted wherever needed.

Xpert® Carba-R test is built on the basis of real time PCR, the targets included in this assay are bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and bla_{OXA-48}. Class A and class B metallo-β-lactamase are identified precisely without any inconsistency. Nevertheless, class D OXA-48 is of high importance because of its low carbapenemase activity and susceptibility to broad spectrum cephalosporins [5]. To resolve this, phenotypic methods cannot be relied upon to detect OXA-48 and it requires molecular characterisation.

The Xpert® Carba-R to identify OXA-48 like variant. Among the 10 known OXA-48 like variants (this includes OXA-48, OXA 54, OXA 162, OXA 163, OXA 181, OXA 199, OXA 204, OXA 232, OXA 242 and OXA 247) [6] manufacturers acknowledge only four variants (bla_{OXA-48}, $bla_{OXA-162}$, $bla_{OXA-163}$ and $bla_{OXA-204}$) [7] were designed to identify, this does not include OXA-181. Incorporating, OXA-181 into Xpert® Carba-R may improve its sensitivity. Probably, manufacturers may include an array of OXA-48 variants specific gene targets in future for accurate detection.

Currently, OXA-181 is the common enzyme variant among the OXA 48-like after to the predominant NDM seen across India [Table/Fig-3]. However IMP, VIM, and KPC are less commonly found among the genes coding for carbapenem resistance. Further, OXA-181 has been identified on the self-conjugative plasmid with the insertion sequence ISEcp1. This insertion sequence promotes the acquisition of ESBL genes and co-production of OXA-181 with other β-lactamases [8-10]. This plasmid borne resistance transmission is a worrisome, especially for highly populated, limited resource countries with poor sanitation and health hygiene.

This study demonstrates the need for geographically-specific knowledge on common resistant mechanisms that code carbapenemase production. Inclusions of prevalent carbapenem resistant gene sequence in the panel of Xpert® Carba-R improve its sensitivity. It is simple to perform and would be useful for the prompt detection and isolation of patients infected or colonized with strains that may harbour carbapenemase genes.

S. No	Organism	No. of isolate included	No. of carbapenem resistant isolates	Study type	Study conducted period	Sequencing of genes	OXA-48 and its variants reported (No.)	Co-producers (No.)	Remarks	Reference
1	*Enterobacteriaceae*	1,443	26	Multicenter SENTRY surveillance program	2006-07	Yes	OXA-181 (10)	CTX-M-15 (10) VIM-5 (1)	All the ten OXA-181 were identified from *K. pneumoniae*	[11]
2	*Enterobacteriaceae*	235	66	SMART study	2009	Yes	OXA-48 (3)	CTX-M-15 (3)	Only 3 isolates with OXA-48 are reported from India	[12]
3	*Enterobacteriaceae*	111	111	Single centre	2010	Yes	OXA-181 (2)	-	Reported in *K. pneumoniae* and C. freundii	[13]
4	*Escherichia coli*	300	45	Single centre	2012	Yes	OXA-48 (25)	NDM-1 (25)	Only *E. coli* is included in the study	[14]

[Table/Fig-3]: Prevalence of OXA-48 in India

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.
2. Associate Research Officer, Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.
3. Associate Research Officer, Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.
4. Associate Research Officer, Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.
5. Professor and Head, Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Balaji Veeraraghavan,
Professor and Head, Department of Clinical Microbiology, Christian Medical College,
Vellore – 632 004, Tamil Nadu, India.
E-mail: vbalaji@cmcvellore.ac.in

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