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SHORT REPORT

Rapid Carbapenemase Detection With Xpert Carba-R V2 Directly On Positive Blood Vials

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Aurélie Cointe[1,2]
Violaine Walewski[1,3]
Claire Amaris Hobson[1]
Catherine Doit[1,2]
Philippe Bidet[1,2]
Laurent Dortet[4–6]
Stéphane Bonacorsi[1,2]
André Birgy[1,2]

[1]IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, AP-HP, Service de Microbiologie, Hôpital Robert-Debré, AP-HP, Paris, France; [2]Service de Microbiologie, Centre National de Référence associé Escherichia coli, Hôpital Robert-Debré, AP-HP, Paris, France; [3]Service de Microbiologie, Hôpitaux Universitaires de Paris Seine Denis (HUPSSD), site Avicenne, AP-HP, Bobigny, France; [4]EA7361, Université Paris-Sud, Université Paris-Saclay, LabEx Lermit, Service de Bactériologie-Hygiène, Hôpital Bicêtre, AP-HP, Le Kremlin-Bicêtre, France; [5]Centre National de Référence associé de la résistance aux antibiotiques: Entérobactéries productrices de carbapénémases, Le Kremlin-Bicêtre, France; [6]Evolution et Ecologie de la résistance aux antibiotiques, Institut Pasteur - APHP -Université Paris Sud, Paris, France

Correspondence: André Birgy
Service de Microbiologie, Hôpital Robert Debré, 48 Boulevard Serrurier Paris F75019, France
Tel +33140034060
Fax +33140032450
Email andre.birgy@aphp.fr

Abstract: The rapid detection of carbapenemase allows implementation of infection control measures and adaptation of antibiotic therapy. We evaluated the performances of the Xpert Carba-R V2® assay for the direct detection and identification of carbapenemase on positive blood cultures. We focused our evaluation on its detection capacity and on the risks of interference due to the patient's blood. Isolates of several variants of OXA-48-like (n=10), KPC (n=10), NDM (n=11), VIM (n=7), IMP-1 (n=1) carbapenemases and 14 non carbapenemase-producing *Enterobacteriaceae* were tested. For each isolate (n=53), an aerobic vial was seeded, and incubated in Bactec Fx (Becton Dickinson®) automate. When positive, the Xpert® Carba-R-V2 assay was assessed for carbapenemase detection using 40 µl aliquot. Reproducibility tests were performed on a subset of 23 isolates using aerobic and anaerobic vials. Longer incubation time was also evaluated on 6 isolates. A complementary prospective study in real-time testing of patient-derived clinical samples on 20 additional positive blood vials with Gram negative bacilli on direct examination was performed. Perfect sensitivity and specificity (100%) were observed regardless of the carbapenemase type, the blood vials used and the time of incubation. Xpert® Carba-R-V2 assay is suitable for the rapid detection of the main carbapenemase genes directly on positive blood vials. Its performances and rapid time analysis allow its use in routine to guide therapeutic choices and to implement infection control measures.

Keywords: carbapenemase, rapid detection, positive blood culture, GeneXpert, antibiotic therapy, infection control measures

Introduction

The rapid dissemination of carbapenemase-producing (CP) *Enterobacteriaceae* (CPE) throughout the world is worrisome and threatens public health. Early detection of patients carrying and/or infected by a CP isolate is a real challenge to implement infection control measures, which limit the risk of hospital spread, and to adapt the antibiotic therapy in case of infection, as it has been shown to improve the patients' survival.[1]

The prevalence and distribution of CP types and variants vary geographically.[2] These enzymes are organized in 3 classes according to the Ambler classification: class B metallo-β-lactamases; including IMP, VIM, and NDM; class A β-lactamases such as *Klebsiella pneumoniae* carbapenemase (KPC) and the class D β-lactamase OXA-48 and variants.

A variety of tests are commercialized to detect carbapenemase activity or the genes encoding these proteins (colorimetric-based assays,[3] phenotype-based assays,[4,5] Matrix Assisted Laser Desorption Ionisation - Time of Flight (MALDI-TOF)

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carbapenem hydrolysis assays,[6] immunochromatographic assays[7] and PCR-based method[8,9] but only a few of them have been evaluated directly on biological samples.

Fully automated PCR-based tests, which provide result in less than an hour, have been developed in recent years making this method attractive. Among these, the Xpert® Carba-R V2 (Cepheid, Sunnyvale, CA 94.089 United States) is a real-time PCR assay for rapid detection and differentiation of 5 genes (bla_{KPC}, bla_{VIM}, bla_{IMP-1}, bla_{NDM} and bla_{OXA-48} and variants) responsible for carbapenem resistance in *Enterobacteriaceae*. It is "European Conformity In Vitro Diagnostics (CE IVD)" marked to detect fecal colonization for surveillance and screening purposes, with excellent sensitivity and specificity,[8] and it is also validated for polymicrobial specimens such as abdominal drainage fluid and bronchial specimens.[10,11]

In this work, we evaluated the performances of the Xpert® Carba-R V2 (Cepheid, Sunnyvale, CA 94.089 United States) assay for the detection of CPE directly on positive blood cultures. We focused our evaluation on its detection capacity and on the risks of interference due to the patient's blood by testing several vials for the same isolate.

Materials And Methods

Isolates

We tested previously characterized variants of OXA-48-like (n=10), KPC- (n=10), NDM- (n=11), IMP-1 (n=1) and VIM-type (n=7) CPE[5,7] and 14 non-CPE (Table 1).

Blood Vials And Sample Preparation

Isolates were cultured from frozen stock on Trypticase-Soy agar (Biomérieux, Marcy l'Etoile, France) at 35°C for 18h. First, from an isolated colony, a suspension of 10^4 CFU/mL was prepared in sterile water. For each isolate (n=53), we inoculated 1 mL of this suspension in an Aerobic/F vial (Becton Dickinson, Franklin Lakes, New Jersey, United States) containing human blood, which was then incubated in the BactecFx until positive. To approach the clinical conditions, we used the vials that remained negative after 5 days of incubation, which we anonymized. Our study was conducted in a Paediatric Hospital hence the volumes of blood in the samples were variable. To evaluate the impact of blood composition, each vial selected corresponded to a different patient.

Second, at positivity, a 40 µl aliquot (or 400 µl aliquot during the development step) was directly mixed with Sample Reagent Buffer and the cartridge was launched with 1.7 mL of this mix.

Third, to test the reproducibility of the test on different blood samples, we seeded and evaluated for carbapenemase detection 2 further vials (1 Plus Aerobic/F and 1 Lytic/10Anaerobic/F) per selected isolate (CPE OXA-48-like (n=5), KPC (n=5), NDM (n=5), IMP (n=1), VIM (n=5) and 2 non-CPE), corresponding to 46 supplementary vials. Then, as in some healthcare centers, positive blood vials during the night are only tested the next morning, we selected 1 isolate per carbapenemase type and 1 non-CP isolate for which the 3 vials (2 aerobic and 1 anaerobic) were re-incubated after positivity for 24h at 35°C before repeating the test (18 vials in total). Finally, in order to approach the clinical conditions, a complementary prospective study in real-time testing of patient-derived clinical samples on 20 additional positive blood vials with Gram negative bacilli on direct examination was performed.

We analyzed the inoculum by plating bacterial dilutions onto TSA and quantified the colonies after 18 h of incubation. For all the samples, a minimum of 10^7 CFU/mL was observed.

Results

A total of 137 blood vials were tested in different conditions including 73 Aerobic vials as soon as they were positive (corresponding to 53 different isolates and the 20 vials for the prospective study), 23 Aerobic and 23 Anaerobic vials to test the reproducibility of the test with different patients' blood and 18 (12 Aerobic and 6 Anaerobic) to repeat the test after 24h of further incubation. We also evaluated the impact of the aliquot volume, and for that, we experienced using 400 µl aliquot and a hemolysis step. In this latter experience, the detection of carbapenemase genes with the Xpert® Carba-R V2 assay (Cepheid, Sunnyvale, CA 94089 United States) was correct for 21/24 tested positive blood vials (87.5%). The 3 remaining (15%) led to errors in the reaction on the GeneXpert device (error 2008 related to syringe pressure due to hyperviscous samples). Further tests (results not shown) showed that hemolysis was not mandatory with 40 µl samples.

Whatever the tested conditions, no further error occurred when using the 40 µl aliquot, and all the positive blood vials seeded with CP isolates were correctly characterized either at time of positivity or after 24h of further incubation. The 14 non-CP isolates were tested negative.

Table 1 Results Of The Xpert Carba-R V2® Assay Performed On Positive Blood Cultures Seeded With Carbapenemase-Producing *Enterobacteriaceae*

Carbapenemase Type	Isolates	Carbapenemase Gene	Other Betalactamases Genes	Total Number Of Tested Vials	Positive Xpert® Carba-R Assay	Sensitivity (%)	Specificity (%)
OXA-48 and variants (n=10)	*E. coli*	OXA-48	-	23	23	100	100
		OXA-181	CTX-M-15, TEM-1, OXA-1				
	K. pneumoniae	OXA-48	CTX-M-15, TEM-1, OXA-1				
		OXA-48	CTX-M group 1, TEM-1, OXA-1				
		OXA-244	CTX-M group 9, TEM-1				
	K. oxytoca	OXA-181	-				
		OXA-48	-				
	C. koseri	OXA-181	-				
	C. freundii	OXA-48	-				
	E. cloacae	OXA-48	-				
KPC (n=10)	*E. cloacae*	KPC-2	TEM, OXA-9	23	23	100	100
		KPC-3	TEM-1, OXA-1				
	E. aerogenes	KPC-2	TEM-1, SHV-2				
	E. coli	KPC-2	CTX-M group 1, TEM				
	K. pneumoniae	KPC-2	TEM				
		KPC*	-				
		KPC-2	TEM-1				
		KPC-2	CTX-M group 1, TEM				
		KPC*	CTX-M group 1, TEM				
		KPC*	TEM				
NDM (n=11)	*E. coli*	NDM-1	CTX-M-15, TEM-1	24	24	100	100
		NDM-5	TEM-1, OXA-1				
		NDM*	TEM, SHV, OXA-1				
		NDM*	OXA-1				
	P. mirabilis	NDM-1	CTX-M group 1				
	C. freundii	NDM-1	TEM, SHV, OXA-1				
	C. farmeri	NDM-1	CTX-M group 1				
	E. cloacae	NDM*	-				
	K. pneumoniae	NDM-1	CTX-M-15, TEM-1, OXA-1				
		NDM-5	CTX-M group 1				
		NDM-1	CTX-M group 1				

(*Continued*)

Table 1 (Continued).

Carbapenemase Type	Isolates	Carbapenemase Gene	Other Betalactamases Genes	Total Number Of Tested Vials	Positive Xpert® Carba-R Assay	Sensitivity (%)	Specificity (%)
VIM (n=7)	*E. coli*	VIM-1	TEM, OXA-1	20	20	100	100
		VIM-1	TEM				
	P. stuartii	VIM-1	SHV				
	C. farmeri	VIM*	-				
	K. pneumoniae	VIM-1	-				
		VIM-4	-				
	P. mirabilis	VIM-1	-				
IMP-1 (n=1)	*K. pneumoniae*	IMP-1	-	6	6	100	100
Non carbapenemase (n=14)	*E. coli*	-	TEM, SHV, OXA-1	21	0	-	100
		-	CTX-M group 1, TEM-1, OXA-1				
		-	SHV-2a				
		-	SHV-12				
		-	CTX-M-15, OXA-1				
		-	CTX-M-2, TEM-1				
		-	CTX-M-14				
	K. pneumoniae	-	CTX-M group 1, TEM-1, OXA-1				
		-	CTX-M group 1				
		-	CTX-M-15, TEM-1, SHV-11				
		-	CTX-M group 9				
		-	CTX-M group 1				
	E. cloacae	-	Derepressed AmpC				
		-	CTX-M group 1, TEM-1, OXA-1				

Notes: *Variants not determined; -none.

For the prospective study, all tests were negative (n=20) and were consistent with the phenotypic susceptibility testing. These negative results were predictable due to the still low prevalence of carbapenemase-producing *Enterobacteriaceae* in France.[12] Nevertheless, they show that this test is applicable in real-time in clinical laboratories. Thus, a sensitivity and specificity of 100% were observed, whatever the blood vials used and the duration of the incubation. The main results are summarized in Table 1.

Discussion

We evaluated the Xpert® Carba-R V2 (Cepheid, Sunnyvale, CA 94089 United States) CPE detection method, directly on positive blood vials, that allows the detection and identification of the main carbapenemase encoding genes in less than one hour whereas most CPE detection techniques require incubation times of several hours to reveal carbapenems hydrolysis.[13,14] Rapid and accurate identification of CPE types can guide therapies choices and impact patient outcomes.[15]

Jaureguy et al reported good performances[16] of this test on aerobic vials (Becton Dickinson, Franklin Lakes, New Jersey, United States) inoculated with CP isolates, but which did not contain blood. In our study, to approach clinical conditions, we focused on assessing the influence of the patient's blood on the performances of the test. Both the sensitivity and the specificity of the test were of 100%. When increasing the aliquot volume (400 µL) and using hemolyzed blood, some results were misleading probably due to the viscosity of samples (possibly dependent on the red blood cells or leukocytes count), indeed, all tests were valid after reducing the aliquot volume to 40 µL.

Only few assays allow working directly on positive blood culture vials. Among these, the FilmArray® (Biomérieux, Marcy l'Etoile, France) can, up to now, only detect bla_{KPC}. The Immunochromatographic RESIST-4 O.K.N.V assay (Coris®, CorisBioconcept, Belgium) is rapid (15min) but shows a limited sensitivity on class B carbapenemases.[7] GenMark's ePlex Blood Culture Identification (BCID) panels can detect bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{OXA-48} genes with results obtained in 1.5 hrs, but it is more expensive than the GeneXpert Test.

We identified some limitations in our work. As we privileged the diversity of type of vials (aerobic and anaerobic), the time of incubation and the source of blood, we only tested a limited number of strains and variants of carbapenemase. Furthermore, due to local epidemiology, only 1 IMP-1 isolate was available for this study and all tests conducted in prospective were negative[12,17]

Conclusion

Xpert® Carba-R assay (Cepheid, Sunnyvale, CA 94089 United States) is suitable for the rapid and easy detection of the main carbapenemase genes using the positive blood vials directly. Considering its performances and the rapid analysis, this test is a useful tool not only for therapeutic decision but also to implement hygiene measure precautions. Accordingly, this test respond to most of the "ASSURRED" criteria described by the World Health Organization and defined by affordable, high sensitivity and specificity, user friendly, rapid, easy and long shelf life tests. However, it should be remembered that the GeneXpert test only detects the 5 main types of carbapenemases (bla_{KPC}, bla_{VIM}, bla_{IMP-1}, bla_{NDM} and bla_{OXA-48} or some variants). Therefore, local epidemiology should be taken into account when using this test.

Ethics

All procedures performed in this study were in accordance with, and with approval from the University Hospital Robert Debré's local ethics committee and with the Helsinki Declaration.

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Disclosure

The author reports no conflicts of interest in this work. More specifically, they have no association with Cepheid.

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