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Detection of Carbapenemase-Producing *Enterobacteriaceae* in Positive Blood Culture Using an Immunochromatographic RESIST-4 O.K.N.V. Assay

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ABSTRACT We evaluated the performance of the RESIST-4 O.K.N.V. assay (Coris) with 98 isolates to detect OXA-48-like and KPC-, NDM-, and VIM-type carbapenemases directly on positive human blood cultures. OXA-48-like and KPC-type isolates were correctly detected, but the detection of NDM- and VIM-type carbapenemases was weak and variable. We show that repeating the test on a 4-h subculture improves the detection of NDM- and VIM-type carbapenemases to 100%.

KEYWORDS *Enterobacteriaceae*, carbapenemase detection, direct detection, immunochromatographic test, positive blood vials, rapid

Rapid detection and characterization of carbapenemase type according to the Ambler classification of class A (e.g., KPC- and GES-type), class B (e.g., NDM and VIM-type), and class D (OXA-48 and variants) lead to improved patient care and guide the implementation of infection control measures. The delay in effective treatment has a direct impact on bacterial infection mortality; thus, the use of a rapid response test for specimens, such as blood culture, is important (1).

We evaluated the performance of the new immunochromatographic RESIST-4 O.K.N.V. assay (Coris) (2, 3) to detect OXA-48-like and KPC-, NDM-, and VIM-type carbapenemases on positive blood cultures. Contrary to other studies (4, 5), we used the Becton, Dickinson Bactec culture vials (Aerobic/F and Lytic/10 Anaerobic/F) with human blood. To create conditions close to the clinical conditions, we used the vials that remained negative after 5 days of incubation, which we anonymized secondarily. Our study was conducted in a pediatric hospital, and the volumes of blood in the samples are often variable; hence, we reproduced these conditions. To evaluate the impact of blood composition, we selected each vial corresponding to a different patient.

A total of 98 characterized isolates of several variants of OXA-48-like ($n = 30$); KPC-type ($n = 17$), NDM-type ($n = 25$), and VIM-type ($n = 8$) carbapenemases and non-carbapenemase-producing *Enterobacteriaceae* (of different beta-lactamase types) ($n = 18$) were selected for this work (see Table S1 in the supplemental material) (6, 7). For each isolate, an aerobic vial was seeded and evaluated for carbapenemase detection when positive. Furthermore, to assess the repeatability of the test with different blood vials, we seeded and evaluated 2 more vials (1 aerobic and 1 anaerobic) for 5 isolates of each type (OXA-48-like; KPC-, NDM-, and VIM-type carbapenemases; and non-carbapenemase-producing *Enterobacteriaceae*) for carbapenemase detection.

Isolates were cultured from frozen stock on Trypticase soy agar (TSA; bioMérieux, Marcy l'Etoile, France) at 35°C for 18 h. From an isolated colony, we suspended 10^4

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CFU/ml in sterile water, and 1 ml of this suspension was inoculated in the Bactec blood vials and incubated until positive. 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. Not only does this result match the previously reported bacterial loads (2.10^7 to 7.10^9 CFU/ml) standardly found in positive blood vials (8), but it is also above the Coris assay's detection limit of 10^6 CFU/ml, which has been described in several studies (5, 9). From these positive vials, two protocols were evaluated. The first protocol was performed directly on the positive blood vial. A 1.8-ml aliquot was collected and centrifuged at 14,000 rpm for 90 s. After discarding the supernatant, the pellet was resuspended in 1 ml of distilled water for 1 min for hemolysis before centrifugation with the same parameters. This red blood cell lysis stage permitted an increase in the intensity of the bands, particularly for VIM- and NDM-type carbapenemases (data not shown). The supernatant was discarded, 40 μl of the residue was mixed with 12 drops of lysis buffer A, and 3 drops of this suspension was added on the 2 zones of deposit on the O.K. and N.V. strips. The second protocol was performed on a 4-h subculture on Polyvitex (PVX) agar (bioMérieux) under aerobic conditions at 35°C. A 1-μl loop of bacteria was mixed with 12 drops of lysis buffer A, and, as described previously, 3 drops of this suspension was added on the zones of deposit on the strips. The results were read at 15 min by two independent blinded readers.

We demonstrated that 100% of OXA-48-like (30/30) and KPC-type (17/17) isolates were correctly detected by the RESIST-4 O.K.N.V. directly on the positive blood vials (Table 1). In contrast, we could only detect 28% (7/25) of NDM-producing isolates, which is equivalent to the weak detection level reported by Wareham et al (4), and 75% (6/8) of VIM-producing isolates with this protocol.

The repeatability test (evaluated on 5 isolates of each type and 2 different vials per isolate) showed similar results, with a perfect detection of both OXA-48-like and KPC-type carbapenemases but only 20% and 70% detection for NDM- and VIM-type carbapenemases, respectively. When positive, the NDM and VIM lines were weak but detectable (confirmed by 2 independent readers). As the inoculum for all tested blood vials was higher than the reported detection limit, poor performances in the detection capacity of NDM and VIM types were not due to variations in this parameter. The test was negative for all non-carbapenemase-producing *Enterobacteriaceae*.

The 4-h subculture on PVX agar allowed a correct detection for 100% of OXA-48-like, KPC-type, NDM-type, and VIM-type isolates under all tested conditions. Overall, as the non-carbapenemase isolates gave a negative result and no cross-reactivity was observed with nontargeted carbapenemases, the specificity was 100% regardless of the testing conditions.

An evaluation of this test on human blood vials from different patients showed an excellent detection capacity for OXA-48-like and KPC-type carbapenemases. No differences were observed regarding the blood vials used. In contrast, the variability and low detection capacity of VIM- and NDM-producing *Enterobacteriaceae* led us to evaluate an alternative test performed on a 4-h subculture. These results confirm the detection of carbapenemase production on positive blood vials in a few hours and vindicate the routine use of this test.

In conclusion, we suggest testing carbapenemase production either directly on blood vials after red blood cell lysis in countries where OXA-48-like or KPC are the leading carbapenemase types and/or on a 4-h culture on PVX agar in countries where epidemiology is more diverse or in which the B class is predominant.

Not only is this method a pertinent test for carbapenemase detection, but also, when used on clinical samples, it can be considered a complementary tool for the therapeutic management of patients and the implementation of infection control measures in health care institutions.

TABLE 1 Results of RESIST-4 O.K.N.V. assay performed on positive blood cultures seeded with carbapenemase and non-carbapenemase-producing *Enterobacteriaceae*

Carbapenemase type (*n*)	Species	Beta-lactamase gene(s)	No. of isolates	No. of positive RESIST-4 O.K.N.V. assay result on:		Sensitivity (%)		Specificity (%)	
				Positive vials	4-h subculture	Positive vials	4-h subculture	Positive vials	4-h subculture
OXA-48 and variants (30)	*E. coli*	OXA-48	10	10	10	100	100	100	100
		OXA-181	3	3	3				
	Klebsiella pneumoniae	OXA-48	10	10	10				
		OXA-244	1	1	1				
	Enterobacter cloacae	OXA-48	2	2	2				
	Klebsiella oxytoca	OXA-48	1	1	1				
		OXA-181	1	1	1				
	Citrobacter freundii	OXA-48	1	1	1				
	Citrobacter koseri	OXA-181	1	1	1				
KPC (17)	*K. pneumoniae*	KPC-2	13	13	13	100	100	100	100
	E. cloacae	KPC-2	1	1	1				
		KPC-3	1	1	1				
	Enterobacter aerogenes	KPC-2	1	1	1				
	E. coli	KPC-2	1	1	1				
NDM (25)	*K. pneumoniae*	NDM-1	7	2	7	28.0	100	100	100
		NDM-5	1	1	1				
	E. coli	NDM-1	5	2	5				
		NDM-5	2	2	2				
	Proteus mirabilis	NDM-1	3	0	3				
	Morganella morganii	NDM-1	2	0	2				
	C. farmeri	NDM-1	2	0	2				
	E. cloacae	NDM-1	1	0	1				
	P. vulgaris	NDM-1	1	0	1				
	C. freundii	NDM-1	1	0	1				
VIM (8)	*E. coli*	VIM-1	3	3	3	75.0	100	100	100
	K. pneumoniae	VIM-1	1	1	1				
		VIM-4	1	0	1				
	P. mirabilis	VIM-1	1	1	1				
	Citrobacter farmeri	VIM-1	1	0	1				
	Providencia stuartii	VIM-1	1	1	1				
Non-carbapenemase producing (18)	*K. pneumoniae*	CTX-M-1 + TEM-1 + OXA-1 + SHV	1	0	0			100	100
		ESBL	1	0	0				
		CTX-M	1	0	0				
		ESBL	1	0	0				
	E. cloacae	CTX-M-1 + derepressed AmpC + TEM-1 + OXA-1	1	0	0				
		CTX-M and derepressed AmpC	1	0	0				
		Derepressed AmpC	1	0	0				
	E. coli	SHV-2a	1	0	0				
		CTX-M-15 + TEM-1	1	0	0				
		SHV-12	1	0	0				
		TEM-52	1	0	0				
		CTX-M-15 + OXA-1	1	0	0				
		CTX-M-2 + TEM-1	1	0	0				
		CTX-M-14	1	0	0				
		CTX-M-3	1	0	0				
		CTX-M-1 + TEM-1 + OXA-1	1	0	0				
		TEM-1 + SHV-1 + OXA-1	1	0	0				
		CTX-M-1	1	0	0				

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01828-18.

SUPPLEMENTAL FILE 1, XLSX file, 0.01 MB.

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We declare no conflicts of interest.

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