Rapid detection of the main carbapenemases in Brazil directly from spiked blood culture using the RESIST-3 O.K.N. immunoassay

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Abstract

The emergence of carbapenem-resistant *Enterobacterales* (CRE) is a matter of public health concern. Carbapenemases are the main mechanism of resistance among CRE, and its rapid detection is essential. The detection of carbapenemases usually requires culture-based methods and molecular assays, which may be costly and need long turnaround times. Recently, an easy and rapid immunochromatographic assay for carbapenemases (OXA-48, KPC, and NDM) detection based in lateral flow immunoassay with specific monoclonal antibodies on a nitrocellulose membrane has been developed. We aimed to evaluate the RESIST-3 O.K.N. in colonies from pure culture as well as in spiked blood cultures with *Enterobacterales*. All carbapenemase producers (CP) presenting the OXA-48-like, KPC, and NDM enzymes presented positive results in both pure colonies and spiked blood cultures. None of the carbapenemase non-producers (CNP) presented positive results in the tests. A total of 97% CP isolates presented positive results in pure colonies in less than 5 min. For CP directly from blood culture, the mean time to positivity for OXA-48-like and KPC was 1 min, whereas it was 25 min for NDM. Our results indicate that this immunoassay can be used to detect carbapenemases directly from blood culture bottles in a routine diagnostic laboratory, which would reduce the turnaround time of CP detection.

Keywords Carbapenemase · Multiplex immunochromatographic assay · Rapid diagnostic · RESIST-3 O.K.N.

Introduction

The emergence of carbapenem-resistant *Enterobacterales* (CRE) is a matter of public health concern, which seriously compromises antibiotic treatment for severe infections. The identification, treatment, and control of infections due to

CRE are a major challenge for healthcare institutions and diagnostic laboratories worldwide [1]. Resistance to carbapenems is usually due to the production of carbapenemase enzymes. The detection of carbapenemases is mainly based on the phenotypic (culture-based methods) and genotypic (molecular methods) methods with long turnaround times of identification and often costly [2, 3]. The identification of bacteria causing bloodstream infections as well as its antimicrobial susceptibility by conventional bacteriological culture takes around 48 h after the blood culture becomes positive, and for this reason, broad-spectrum antibiotics are usually administered empirically [4].

OXA-48, KPC, NDM, GES, VIM, and IMP are the most common carbapenemases in *Enterobacterales* worldwide, although their prevalence may vary considerably in different regions [5]. In Brazil, the main carbapenemase is KPC; however, there are also some reported cases of infections due to *Enterobacterales* carrying NDM, IMP, VIM, GES, OXA-48-like, and BKC-1 [6, 7]. The OXA-370, an OXA-48 variant, was first described in a carbapenem-resistant *Enterobacter hormaechei* recovered from a rectal swab from a patient from

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Porto Alegre, a city in the southern of Brazil. It was observed that all of the isolates possessed the OXA-370 allelic variant differ from *bla*_{OXA-48} by three nucleotide changes, resulting in one amino acid substitution [8].

Detection of carbapenemases in the routine diagnostic laboratory is challenging, and a gold-standard method has not been established. The large numbers of different genotypes that are present worldwide are associated with many diverse phenotypes, and few, if any, of the detection methods available to routine diagnostic laboratories can detect all carbapenemase types with high sensitivity and specificity [9]. Therefore, the rapid identification of bacteria as well as their resistance profile in the routine microbiology laboratory plays a crucial role in the treatment of patients.

Recently, an easy and rapid detection test for carbapenemases, the RESIST-3 O.K.N. assay (Coris BioConcept, Gembloux, Belgium), which is based on a lateral flow immunoassay with specific monoclonal antibodies on a nitrocellulose membrane has been developed for the simultaneous detection of OXA-48, KPC, and NDM carbapenemase producers directly from bacterial colonies.

The aim of this study was to evaluate the RESIST-3 O.K.N. assay using pure colonies of several species of *Enterobacterales* which are carbapenemase producers (CP) and non-producers (CNP). We also attempted to evaluate the performance of the commercial test to detect CP and CNP directly from spiked blood culture flasks.

Materials and methods

Isolates

All clinical isolates included in this study were obtained from a previous surveillance study of CP in *Enterobacterales* among clinical isolates in southern Brazil from April 2013 to May 2018. All these isolates were submitted to multiplex real-time PCR (RT-PCR) assay with primers for *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{GES}, *bla*_{IMP}, and *bla*_{VIM} [10].

For the assay from pure bacterial colonies, a total of 76 CP isolates of *Enterobacterales* were tested: 29 KPC producers, 21 NDM producers, 10 OXA-48-like producers (OXA-370), 3 GES producers, 1 IMP producer, 1 VIM producer, 5 NDM + OXA-48-like producers, 5 KPC + NDM producers, and 1 KPC + GES producer. Eighteen ($n = 18$) clinical isolates negative for carbapenemase genes were also included (Table 1).

For the assay directly from spiked blood culture, 27 CP isolates were evaluated: 11 KPC producers, 4 NDM producers, 8 OXA-48-like producers (OXA-370), 1 GES producer, 1 VIM producer, 1 IMP producer, and 1 KPC + NDM producer. Ten clinical isolates negative for carbapenemase genes were included (Table 2).

Immunochromatographic assay from bacterial colonies

To evaluate the RESIST-3 O.K.N. assay, the test from pure colonies was carried out according to the manufacturer's protocol. In brief, bacterial colonies from an overnight subculture on Mueller-Hinton agar were suspended in 10 drops of LY-A buffer® provided with the kit. Three drops of this bacterial suspension were added to the sample well of the RESIST-3 O.K.N. cassette. The cassette was incubated at room temperature for up to 15 min. Positive results for OXA-48-like, KPC, and NDM were indicated by a reddish-purple band at the control line and at the specific test positions of the nitrocellulose membrane. Negative results were indicated by reddish-purple line at the control line and absence of any other band.

Immunochromatographic assay directly from spiked blood culture

The RESIST-3 O.K.N. immunoassay was also evaluated on spiked blood cultures with CP isolates. The assay used 9 mL of sterile human blood spiked with 30 μ L of a bacterial inoculum of 10^3 CFU/mL (final concentration) which were added to adult blood cultures flasks (bioMerieux, Durham, England). Spiked blood culture flasks were incubated in an automated system (BacT/ALERT, bioMerieux, NC, USA) until the flasks were flagged positive. A volume of 0.5 mL of the blood culture bottle flagged positive was centrifuged for 2 min at $15,000 \times g$ at room temperature, and the pellet was suspended with 10 drops of LY-A buffer®. Three drops of the bacterial suspension were added to the sample well of the RESIST-3 O.K.N. cassette which was incubated and read as described above.

Results

All isolates with OXA-48-like, KPC, and NDM carbapenemases presented positive results from pure colonies which were clearly indicated by a reddish-purple band at the specific test line positions on the nitrocellulose membrane of the RESIST-3 O.K.N. cassette (Fig. 1). In fact, 97% of the CP presented positive results in less than 5 min. None of the non-carbapenemase producers neither the VIM, GES, nor IMP producers presented positive results in the commercial immunochromatographic assay (Table 1).

Considering the CP spiked in blood cultures, the assay was positive for all OXA-48-like, KPC, and NDM isolates. Due to the presence of the hemoglobin of red blood cells, the observation of the reddish-purple band at the specific test line positions was somehow less clear but sufficient visible (Fig. 1). Most of the OXA-48-like and KPC producers presented positive results in less than 5 min. However, most NDM-positive

Table 1 Results of the RESIST-3 O.K.N. immunoassay for *Enterobacteriales* carbapenemase producer and non-producer colonies from Mueller-Hinton agar

Characteristics of isolates	No. of isolates									
	<i>Citrobacter freundii</i>	<i>Enterobacter sp.</i>	<i>E. aerogenes</i>	<i>E. cloacae complex</i>	<i>E. hormaechei</i>	<i>Escherichia coli</i>	<i>Klebsiella oxytoca</i>	<i>K. pneumoniae</i>		
CP	5	6	1	12	1	8	3	20		
KPC	3	3			1	5	2	11		
OXA-48 like			1	5				2		
NDM	2	2		1		3	1	4		
OXA48like + NDM				5						
KPC + NDM		1		1				3		
KPC + GES										
VIM				1						
GES										
IMP										
CNP	1	3		4		1		1		
Total	6	9	1	17	1	9	3	27		

Characteristics of isolates	No. of isolates									
	<i>Morganella morganii</i>	<i>Pantoea sp.</i>	<i>Proteus mirabilis</i>	<i>Providencia rettgeri</i>	<i>P. stuartii</i>	<i>Serratia marcescens</i>	No. of isolates with positive result RESIST-3 O.K.N.			
CP	4						71/71			
KPC	1	1	2	3	1	4	29/29			
OXA-48 like			1		1		10/10			
NDM	3		1	2		2	21/21			
OXA48like + NDM							5/5			
KPC + NDM							5/5			
KPC + GES				1			1/1			
VIM							0/1			
GES						3	0/3			
IMP							0/1			
CNP	2	1					0/18			
Total	6	2	2	3	1	7				

isolates produced only either a faint line or a line not visible at all in 5 min. In order to allow a better observation of the line among NDM producers, the incubation time of RESIST-3 O.K.N. cassette had to be increased to 30 min. No false positive results were observed for the spiked blood cultures (Table 2). Noteworthy, carbapenemase co-producers presented bands for both carbapenemase types in the cassette.

The RESIST-3 O.K.N. presented 100% of sensitivity and 100% specificity for both pure bacterial colonies and spiked blood cultures.

Discussion

Carbapenem resistance is the main mechanism of resistance among CRE. Detection of carbapenemases in the diagnostic laboratory is challenging. The current methods used to detect carbapenemases are culture-based techniques followed by phenotypic and/or genotypic confirmatory assays which may add costs and turnaround times to the procedure [2, 3]. There are several phenotypic approaches proposed to detect carbapenemases, which are usually based on carbapenem hydrolysis (Carba NP, Blue-CARBA, CIM, etc.) [11–14]. Although some of these methods exhibit high performance for the identification of KPC, IMP, VIM, and NDM, none of them is highly sensitive and specific for detecting OXA-48 producers [11–15]. Moreover, most of the methods described above are time-consuming and may require materials that are difficult to be obtained in clinical microbiology laboratories.

In this study, we found a complete agreement between the carbapenemase identification by RT-PCR and the results obtained with the RESIST-3 O.K.N. immunoassay (Tables 1 and 2). The immunochromatographic assay was able to correctly identify and differentiate OXA-48-like, KPC, and NDM production among all CP species and enzyme variants tested, including those carrying more than one carbapenemase. Moreover, all CNP isolates and the isolates harboring *bla*_{IMP}, *bla*_{VIM}, *bla*_{GES}, and *bla*_{OXA-23} genes presented negative results in the RESIST-3 O.K.N., indicating that the test is highly specific.

The RESIST-3 O.K.N. immunoassay was simple to perform and does not require special laboratory materials or equipment to evaluate pure colonies. According to our results, the immunoassay can also be used to detect CP directly from blood culture. In fact, KPC and OXA-48-like isolates presented clear results which can be visualized in a short period of time (less than 5 min). However, some NDM-positive isolates in blood culture presented faint bands in short incubation and required longer times (around 25 min) to show up clear bands. The latter is still considerably shorter than the standard microbiological

Table 2 Results of the RESIST-3 O.K.N. immunoassay for *Enterobacteriales* carbapenemase producers and non-producers directly from blood culture

Characteristics of isolates	No. of isolates	No. of isolates with positive result RESIST-3 O.K.N.									
		<i>C. freundii</i>	<i>E. aerogenes</i>	<i>E. cloacae</i> complex	<i>E. hormaechei</i>	<i>Enterob. sp.</i>	<i>E. coli</i>	<i>K. oxytoca</i>	<i>M. morganii</i>	<i>P. mirabilis</i>	<i>S. marcescens</i>
CP	1	1		5	1	3	2	1	1	1	24/24
KPC	1			1	1	1	1	5		1	11/11
OXA-48 like	1	1		5				1	1		8/8
NDM						1	1	2			4/4
KPC + NDM						1					1/1
VIM						1					0/1
GES				1						1	0/1
IMP								1			0/1
CNP	1					4	1	3	1		0/10
Total	2	1		6	1	7	3	12	1	2

Fig. 1 Evaluation of the tests results in the RESIST-3 O.K.N. assay. 1–4: positive (1 KPC, 2 NDM, and 3 OXA-48-like) and negative (4) results from pure colonies at Mueller-Hinton agar. 5–8: positive (5 KPC, 6 OXA-

48-like, and 7 NDM) and negative (8) results obtained directly from spiked blood culture

workup, but users of this commercial assay directly from blood culture must be aware of this limitation of the test.

In 2016, Pasteran showed that NDM is a lipoprotein anchored to the outer membrane in Gram-negative bacteria, unlike all other known carbapenemases, which have been characterized as soluble periplasmic enzymes [16]. In this study, they showed that false-negative results for detection of NDM producers could be attributed to membrane anchoring of the enzyme. According to these results, we believe the longer time to detect NDM could be due to the fact that it is a membrane-bound lipoprotein.

A few immunochromatographic lateral flow assays have already been developed for the specific detection of OXA-48 (OXA-48 K-SeT) and KPC-like carbapenemase producers (KPC K-SeT) [17, 18]. They were reported to present 100% sensitivity and specificity when compared with molecular methods for the detection of carbapenemase genes. The OXA-48 K-SeT assay was also evaluated in samples taken directly from positive blood culture bottles and presented very satisfactory results to detect OXA-48 isolates. It is important to mention that although both immunochromatographic assays (OXA-48 K-SeT and the KPC K-SeT) presented reliable results, they were tested individually and not as one unique test to detect concomitantly different carbapenemases. The RESIST-3 O.K.N. assay was developed as a more complete version, being designed for the simultaneous detection of OXA-48, KPC, and NDM-like enzymes using a single disposable cartridge.

It could be considered that RESIST-3 O.K.N. has a limitation in terms of detecting other carbapenemases such as IMP, VIM, and GES producers. However, KPC is the most common carbapenemase in the USA, Colombia, Brazil, Argentina, Italy, Poland, China, Taiwan, Israel, and Greece [11, 19, 20]. To the best of our knowledge, OXA-48 has been not described in Brazil as yet, and the OXA-370, which is an OXA-48 variant, is the only oxacillinase reported among *Enterobacteriales* in this country [21, 22]. One could consider that OXA-370 would not be detected in an immunoassay technique, which uses antibody against OXA-48; however, the RESIST-3 O.K.N. assay detected all OXA-370 tested in this study. Although the assay does not currently detect IMP, VIM, and GES producers, its ability to detect three of the five most prevalent and transmissible carbapenemases is a significant advantage for the in vitro detection of these enzymes. Therefore, this test is very appropriate to be used in routine laboratories worldwide.

Our results confirm that the RESIST-3 O.K.N. assay is an accurate method for rapid identification of carbapenemases directly from pure colonies. Moreover, this assay was also very reliable to detected OXA-48-like, KPC, and NDM carbapenemases directly from spiked blood cultures. In fact, OXA-48-like, KPC, and NDM carbapenemases can be reliably detected directly from positive blood culture bottles within 20–30 min, and this can help to rapidly identify patients with CP and optimize their treatment as well as minimize the spread of carbapenemases in the hospital environment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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