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Evaluation of the New Multiplex Immunochromatographic O.K.N.V. *K*-SeT Assay for Rapid Detection of OXA-48-like, KPC, NDM, and VIM Carbapenemases

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The worldwide emergence of highly resistant bacteria, such as carbapenemase-producing *Enterobacteriaceae*, is a major public health concern (1). Rapid detection of these highly resistant bacteria and distinction of carbapenemase production from other carbapenem resistance mechanisms, especially the production of derepressed cephalosporinases or extended-spectrum beta-lactamases combined with impermeability, are crucial to implement appropriate hygiene measures to prevent transmission and health care-associated infections and to avoid outbreaks. Although molecular tests are the gold standard for identification of carbapenemase groups, they may be unaffordable for laboratories because of the cost of reagents and the need for specific equipment and/or trained staff. Confirmatory phenotypic inhibitor-based tests (such as the MBL Etest and the modified Hodge test) are available for identification of class A (KPC) and class B (VIM, IMP, NDM) carbapenemases, but they are time-consuming and require at least one additional day following antimicrobial susceptibility testing (AST) results (2, 3). Commercially available rapid phenotypic tests based on the use of a color shift of a pH indicator after carbapenem hydrolysis have been shown to have a good sensitivity and specificity for the detection of carbapenemases (4, 5). However, the color shift is sometimes difficult to interpret, and it does not allow carbapenemase class identification.

As an alternative, immunochromatographic lateral-flow tests (Coris BioConcept, Gembloux, Belgium) targeting some of the most prevalent carbapenemases have been proposed for routine use as a tool for their identification directly from suspected colonies grown on primary plates. These tests allow the detection of such enzymes in less than 15 min using monoclonal antibodies bound to a nitrocellulose membrane. The first test was designed for the detection of OXA-48 carbapenemases, which are the most challenging enzymes to detect for clinical laboratories because of very low carbapenem MICs inducing false-negative phenotypic tests (6). This test was then improved by adding the detection of KPC and subsequently NDM carbapenemases. All the generations of this test have been proved to be highly reliable in simplex (OXA-48 *K*-SeT, KPC *K*-SeT) and multiplex (RESIST-3 O.K.N.) forms: the sensitivity varies between 94.4% and 100%, and the specificity is 100% compared to molecular techniques (7, 8). Recently, a new multiplex version, the RESIST-4 O.K.N.V. test, has been developed. This allows the identification of 4 enzymes thanks to a combined set of two immunochromatographic cassettes: the first targeting KPC and OXA-48-like carbapenemase detection and the second being dedicated to NDM and VIM detection. We report herein the

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TABLE 1 Description of the carbapenemases produced by the strains included in this study and number of positive O.K.N.V. tests

Bacterial isolate	No. of positive tests/no. of strains				
	Total	VIM	OXA-48-like	KPC	NDM
Citrobacter freundii	3/3	0	3/3	0	0
Enterobacter cloacae complex	24/24	22/22 (4 VIM-1, 18 VIM-4)	3/3	0	0
Escherichia coli	11/11	2/2 (2 VIM-1)	3/3	2/2	4/4
Klebsiella oxytoca	2/2	1/1 (1 VIM-1)	0	0	1/1
Klebsiella pneumoniae	17/17	3/3 (2 VIM-1, 1 VIM-4)	2/2	7/7	5/5
Kluyvera ascorbata	1/1	0	0	1/1	0
Proteus mirabilis	0/1	0	0	0	0/1
Providencia stuartii	0/1	0	0	0	0/1
Pseudomonas aeruginosa	9/9	9/9 (6 VIM-2, 3 VIM-4)	0	0	0
Total	67/69	37/37	11/11	10/10	10/12

performance of this new assay to detect the 4 carbapenemases, especially the VIM type, the detection of which is now possible thanks to this latest version of the test.

We retrospectively included 69 Gram-negative bacillus strains (*Enterobacteriaceae*, $n = 60$; *Pseudomonas aeruginosa*, $n = 9$) harboring carbapenemase genes (VIM, $n = 37$; OXA-48-like, $n = 11$; KPC, $n = 10$; NDM, $n = 12$) (Table 1). All isolates were grown on Columbia agar plates (bioMérieux, Marcy l'Etoile, France) over 24 h at 36°C. Identification of the bacteria was performed using the Vitek MS matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry system (bioMérieux). The detection of carbapenemases with the RESIST-4 O.K.N.V. test was performed according to the manufacturer's recommendations. Briefly, a single colony was suspended in the test buffer for protein extraction. Three drops of the extracted solution were then added into the sample well of each of the two cassettes. The result in comparison with that for the control band was read after 15 min. The Xpert Carba-R assay (v2; Cepheid, Sunnyvale, CA, USA) was used as the reference method for carbapenemase identification. In addition, the VIM gene was subtyped thanks to a specific PCR followed by sequencing (9).

The RESIST-4 O.K.N.V. assay correctly identified all strains with carbapenemases except two NDM-positive strains (one *Proteus mirabilis* strain and one *Providencia stuartii* strain), for which the test was negative and which were subsequently confirmed to be NDM-1 positive by a specific PCR and sequencing (10). The test was repeated under different conditions for these two strains using (i) a higher inoculum (5 colonies versus the 1 colony recommended by the manufacturer), (ii) one colony grown on a selective medium (ChromID Carba; bioMérieux), and (iii) one colony harvested around an ertapenem disk. Only the test using the *Providencia stuartii* strain grown on the ChromID Carba plate was positive after 15 min. Of note, the RESIST-3 O.K.N. assay was also negative. Interestingly, the Rapidec Carba NP test (bioMérieux) was positive for both strains, which were not susceptible to any of the carbapenems tested (MICs measured by Etest [bioMérieux], 2 mg/liter and 6 mg/liter for ertapenem for the *Providencia stuartii* and *Proteus mirabilis* strains, respectively; 16 mg/liter and 4 mg/liter for meropenem for the *Providencia stuartii* and *Proteus mirabilis* strains, respectively; and >32 mg/liter for imipenem for both strains). These phenotypic results confirmed that the NDM carbapenemases were expressed and active. The test correctly detected one strain coproducing VIM-4 and OXA-48-like carbapenemases. No false-positive result was observed with this test. Furthermore, the VIM line remained weakly visible after 15 min for 3 *Enterobacter cloacae* strains (harboring VIM-1 [$n = 1$] and VIM-4 [$n = 2$]). The line became sharper after 5 to 10 min of additional incubation. In addition, we observed that a sharper line was observed when using 5 colonies instead of 1 colony, as recommended. We checked, using two *E. cloacae* isolates that did not produce any carbapenemases, that the extension of the incubation time (from 15 to 25 min) or the increase of the inoculum (from 1 to 5 colonies) did not induce unspecific binding of antibodies and, so, false-positive tests.

To the best of our knowledge, this is the first published study evaluating the performance of the new RESIST-4 O.K.N.V. test. The overall sensitivity of this test was 100% for the detection of VIM, OXA-48-like, and KPC carbapenemase-producing strains and 83.3% for the detection of NDM-producing strains. These results are consistent with data reported for the previous versions of the test. There is, however, an exception for NDM carbapenemases. For instance, Glupczynski et al. reported a sensitivity of 100% for the detection of these carbapenemases using the RESIST-3 O.K.N. *K*-SeT assay on a collection of 140 carbapenemase-producing *Enterobacteriaceae* strains (8). However, this study did not include any NDM-producing *Proteus* or *Providencia* strains. Interestingly, Saleh et al. reported a false-negative result when testing an NDM-1-producing *Proteus mirabilis* strain using the RESIST-3 O.K.N. *K*-SeT assay in another study including 63 carbapenemase-producing strains, among which 18 NDM-positive isolates were found; the test was, however, positive using a higher inoculum or when colonies around a carbapenem disk were harvested (11). The data reported herein for both NDM and VIM detection using the RESIST-4 O.K.N.V. assay also suggest that the inoculum or the medium used may impact the result of the test (i.e., line visibility). However, the internal control included in the extraction buffer attests only to the correct migration and not active extraction of the bacterial proteins and the control of the inoculum, which are parameters that may vary depending on the colony size and physical characteristics (e.g., mucoidy). As the performance of the previous versions of the Coris tests for the detection of the three main carbapenemase families (OXA-48-like, NDM, and KPC) has already been extensively described by others, we chose to include a large number of VIM-producing strains. We tested a limited number of VIM types (VIM-1, -2, -4), which, however, account for 90 to 100% of VIM carbapenemases isolated in *Enterobacteriaceae* and *P. aeruginosa* clinical strains in Europe (12, 13). We also chose not to test carbapenemase-nonproducing strains because previous studies reported a specificity of 100%. Of note, another immunoassay, Carba5 (NG Biotech, Guipry, France), was recently developed for the detection of 5 carbapenemases (NDM, KPC, IMP, VIM, and OXA-48-like) and was reported to have 100% sensitivity and specificity (14).

In conclusion, our results demonstrate that the new RESIST-4 O.K.N.V. assay is highly sensitive and specific for the detection of the four main carbapenemases (OXA-48, KPC, NDM, and VIM). However, clinical microbiologists should keep in mind that the performance of this test may be influenced by the inoculum, the medium used, and the bacterial species. This assay can be used as a first-line test for confirmation of carbapenemase production and its identification for *Enterobacteriaceae*, especially for *Escherichia coli* or *Klebsiella* spp., growing on a screening agar medium or with evocative phenotypic AST results. However, for group 3 *Enterobacteriaceae*, such as *Enterobacter* spp., or for *Pseudomonas aeruginosa*, whose carbapenem resistance is more often due to other mechanisms, this kind of assay should be used as a second-line test.

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