EVALUATION OF THE APPLICABILITY OF THE O.K.N.V.I. RESIST-5 AND THE KPC&MBL&0XA-48 DISK TESTS IN A ROUTINE MICROBIOLOGY LABORATORY

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ABSTRACT

Background: The global spread of carbapenemase-producing *Enterobacterales* (CPE) and the increasing emergence of clinical *Enterobacterales* harboring multiple carbapenemases of different molecular classes have prioritized the use of rapid molecular detection methods in routine microbiology laboratories. The aim of this study was to evaluate the applicability of the immunochromatographic O.K.N.V.I. RESIST-5 and the KPC&MBL&0XA-48 disc tests in a clinical microbiology laboratory. **Material and methods:** The tests were performed with 50 CPE belonging to 8 species and producing 7 molecularly characterized carbapenemases. Six of these isolates carried two different carbapenemases. To assess the specificity of the assays, 18 non-carbapenemase-producing but carbapenem-resistant *Enterobacterales* (non-CP CRE) were also included. Both tests were performed from a common overnight culture on Mueller-Hinton agar with inoculum harvested around an ertapenem disk. **Results:** The O.K.N.V.I. RESIST-5 correctly detected all 56 carbapenemases, including KPC-2 in *Klebsiella pneumoniae*; OXA-48 in *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter hormaechei* and *K. pneumoniae*; NDM-1 in *Escherichia coli*, *Morganella morganii*, *E. hormaechei*, *C. freundii*, *S. marcescens* and *K. pneumoniae*; VIM-1 in *Proteus mirabilis*; VIM-4 in *C. freundii* and *S. marcescens*; VIM-86 with and without NDM-1 in *Providencia stuartii*, and NDM-5 with and without OXA-232 in *K. pneumoniae*. The KPC&MBL&0XA-48 disc tests correctly confirmed KPC, OX-48-like and most MBL except VIM in *P. mirabilis*. Furthermore, the combination disc tests failed to detect OXA-48-like in pairs with MBL in *K. pneumoniae*. **Conclusions:** The O.K.N.V.I. RESIST-5 multiplex lateral assay is an excellent test for rapid diagnostic of CPE in routine microbiology laboratories. It is easy to handle and provides results with 100% sensitivity and specificity when an inoculum around ertapenem disc from routine antibiogram was used.

Keywords: *Enterobacterales*, carbapenemase detection, O.K.N.V.I. RESIST-5, KPC&MBL&0XA-48 disc tests

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INTRODUCTION

The dissemination of carbapenemase-producing *Enterobacterales* (CPE) and the increasing emergence of clinical *Enterobacterales* possessing multiple carbapenemases of different molecular classes have prioritized the use of rapid molecular detection methods in the routine microbiology laboratory (1). Another argument is that some of the few available therapeutic options (e.g. ceftazidime-avibactam) are active against certain classes of carbapenemases but inactive against others (2). Furthermore, as has been reported, prompt initiation of adequate therapy appears to be a determining factor in the management of CPE infections (3).

Several methods are currently used in clinical microbiology laboratories to identify and characterize carbapenemase types, such as culture-based phenotyping methods and molecular methods using gene amplification. However, phenotypic assays are time-consuming, whereas the molecular method needs expensive equipment and high expertise.

Recently, a multiplex lateral flow immunochromatographic assay, O.K.N.V.I. RESIST-5 (CORIS BioConcept, Gemblux, Belgium), was

developed to detect the five major carbapenemases (OXA-48-like, KPC, NDM, VIM, and IMP) identified in *Enterobacterales* worldwide (1). The newly introduced immunochromatographic tests detect carbapenemase-specific epitopes using monoclonal antibodies and are a rapid alternative, taking only 15 minutes without additional equipment. Previous studies of various lateral flow assays detecting different spectra of carbapenemases have shown high sensitivity and specificity for OXA-48-like, KPC, and NDM carbapenemases (4,5). Furthermore, the influence of different culture media and antibiotic discs on test sensitivity was analyzed. It was found that the best results were obtained with inoculum harvested around an ertapenem or meropenem disk or from zinc containing agars (4,5).

The aim of this study was to evaluate the applicability of the O.K.N.V.I. RESIST-5 immunochromatographic assay and the KPC&MBL&0XA-48 disc tests in a routine microbiology laboratory.

MATERIAL AND METHODS

This study collection included 50 clinical isolates of CPE with molecularly characterized carbapenemases by using whole genome sequencing or PCR and sequencing and previously reported in our studies (6–9). Of these, 36 isolates belonged to the *Enterobacter* spp. *Serratia marcescens*, *Citrobacter freundii*, *Providencia* spp., and *Morganella morganii* (ESCPM) group. They consisted of 11 VIM-4 producers (10 *S. marcescens* and 1 *C. freundii*), 10 NDM-1 producers (4 *C. freundii*, 3 *S. marcescens*, 2 *M. morganii* and 1 *Enterobacter hormaechei*), 7 OXA-48 producers (5 *E. hormaechei*, 1 *C. freundii* and 1 *S. marcescens*), 6 VIM-86-producing *Providencia stuartii*, and 2 *P. stuartii* producing VIM-86 and NDM-1 (6). Of the remaining 14 study isolates, 6 *Klebsiella pneumoniae* produced NDM-5 and OXA-232, 3 *Escherichia coli* produced NDM-1, 2 *Proteus mirabilis* produced VIM-1, 1 *K. pneumoniae* was NDM-1 producer, 1 *K. pneumoniae* was OXA-48 producer, and 1 was *K. pneumoniae* KPC-2 producer (7–9). Eighteen non-carbapenemase-producing but carbapenem-resistant *Enterobacterales* (non-CP CRE) were also included to assess the specificity of the assays. This group consisted of 8 *E. hormaechei*, 4 *K. pneumoniae*, 3 *E. coli*, 2 *S. marcescens* and 1 *Klebsiella aerogenes* and was characterized in our previous study (7).

To evaluate the applicability of O.K.N.V.I. RESIST-5 and the KPC&MBL&0XA-48 disc kit for detection and confirmation of carbapenemases in a routine microbiology laboratory, both tests were performed from a common overnight culture on Mueller-Hinton agar (MHA) for each isolate. KPC&MBL&OXA-48 disc tests (Liofilchem, Roseto degli Abruzzi, Italy) were performed as described in our previous study (10). EDTA, cloxacillin, or phenylboronic acid were used as β-lactamase inhibitors with meropenem in combination disc tests for phenotypic differentiation of metallo-β-lactamases, KPC, or AmpC. Results were interpreted according to the manufacturer's recommendations. Immunochromatographic O.K.N.V.I. RESIST-5 assay was performed according to the manufacturer's instructions. Briefly, colonies were harvested around an ertapenem disc from the MHA plate, resuspended in LY-A buffer, and then transferred to the lateral flow test. One assay contained two lateral flow cassettes, one to identify NDM, KPC and OXA-48-like, and the other for IMP and VIM. A positive result is indicated by a red band next to the letters of each carbapenemase. In addition, the minimum inhibitory concentrations (MICs) of meropenem, ertapenem and imipenem were determined by broth microdilution using the MicroScan NM-EN52 panel (Beckman Coulter, Inc., Brea, CA, USA) and the Micronaut-S MDR plate (Merlin Diagnostika GmbH, Bornheim, Germany) by following the manufacturer's protocols. Susceptibility testing results were interpreted in accordance with EUCAST clinical breakpoints v14.0 (11). *E. coli* ATCC 25922 was used for quality control.

RESULTS

All 56 carbapenemases present in the 50 CPE isolates were correctly identified with the O.K.N.V.I. RESIST-5 assay (Table 1, Figures 1 to 5). Of the 50 carbapenemase producers, six isolates expressed two different carbapenemases and all were correctly detected (Table 1, Figures 4b and 5b). All non-CP CRE were negative in the tests. Overall, the sensitivity and specificity of O.K.N.V.I. RESIST-5 for detecting carbapenemases were both 100%. Regarding the KPC&MBL&OXA-48 disc tests, the results of this study confirm our previous findings for VIM MBLs

Table 1. Results from the O.K.N.V.I. RESIST-5, KPC&MBL&OXA-48 disk tests and carbapenem susceptibility testing for 50 CPE with molecularly characterised carbapenemases

Isolate (reference no.)	Year of isolation	Species	Car-bapene-mase(s)	MIC values (mg/L)[a] for:			KPC&MBL&OXA-48 disc tests[b]						O.K.N.V.I. RESIST-5 results
				MEM	ETP	IPM	TMO	MRP	MR+CL	MR+BO	MR+ED	Interpreta-tion	
KP746-PR3337 (9)	2017	*K. pneumoniae*	KPC-2	>128	>1	>8	8	6	6	16	6	KPC	KPC
KP1335-PR2899 (8)	2014	*K. pneumoniae*	OXA-48	0.5	>1	1	6	22	22	23	22	OXA-48-like	OXA-48-like
CF1843 (6)	2014	*C. freundii*	OXA-48	2	>1	2	6	22	22	22	22	OXA-48-like	OXA-48-like
SM585 (6)	2016	*S. marcescens*	OXA-48	4	>1	8	6	21	21	23	21	OXA-48-like	OXA-48-like
EH1401 (6)	2018	*E. hormaechei*	OXA-48	4	>1	2	6	21	21	21	21	OXA-48-like	OXA-48-like
EH3113 (6)	2018	*E. hormaechei*	OXA-48	4	>1	4	6	20	20	20	20	OXA-48-like	OXA-48-like
EH3371 (6)	2018	*E. hormaechei*	OXA-48	2	>1	2	6	22	22	22	22	OXA-48-like	OXA-48-like
EH273 (6)	2020	*E. hormaechei*	OXA-48	2	>1	2	6	23	23	23	23	OXA-48-like	OXA-48-like
EH1872 (6)	2020	*E. hormaechei*	OXA-48	2	>1	2	6	23	23	23	23	OXA-48-like	OXA-48-like
EC3517 (7)	2012	*E. coli*	NDM-1	32	>1	8	6	7	7	9	23	MBL	NDM
MM4395 (6)	2018	*M. morganii*	NDM-1	2	>1	13	20	22	22	24	27	MBL	NDM
SM4015 (6)	2018	*S. marcescens*	NDM-1	32	>1	>8	11	15	15	16	23	MBL	NDM
SM4949 (6)	2018	*S. marcescens*	NDM-1	128	>1	>8	6	6	6	9	23	MBL	NDM
SM4487 (6)	2019	*S. marcescens*	NDM-1	64	>1	>8	6	6	6	8	22	MBL	NDM
EH10088 (6)	2020	*E. hormaechei*	NDM-1	32	>1	8	10	14	15	17	24	MBL	NDM
CF4015 (6)	2018	*C. freundii*	NDM-1	16	>1	8	9	15	16	17	23	MBL	NDM
EC52491 (7)	2018	*E. coli*	NDM-1	16	>1	8	14	16	16	18	27	MBL	NDM
EC52492 (7)	2018	*E. coli*	NDM-1	8	>1	4	14	17	17	19	27	MBL	NDM
CF1976 (6)	2021	*C. freundii*	NDM-1	32	>1	>8	11	14	15	16	21	MBL	NDM
CF2068 (6)	2021	*C. freundii*	NDM-1	16	>1	8	12	15	15	17	23	MBL	NDM
CF2341 (6)	2021	*C. freundii*	NDM-1	16	>1	8	12	15	15	17	22	MBL	NDM
MM231 (6)	2023	*M. morganii*	NDM-1	2	>1	8	20	22	22	24	27	MBL	NDM
KP740 (7)	2023	*K. pneumoniae*	NDM-1	64	>1	8	8	12	12	14	24	MBL	NDM
KP166 (7)	2023	*K. pneumoniae*	NDM-5	64	>1	>8	10	10	10	12	22	MBL	NDM
KP3112 (7)	2023	*K. pneumoniae*	NDM-5	64	>1	>8	8	6	6	6	20	MBL	NDM

[a] MIC, Minimum inhibitory concentration; MEM, meropenem; ETP, ertapenem; IMP, imipenem

[b] mm sone diameters KPC&MBL&OXA-48 disc kit; TMO, temocillin; MRP, meropenem; MR+CL: meropenem+cloxacillin; MR+BO, meropenem+phenylboronic acid; MR+ED, meropenem+EDTA

Isolate (reference no.)	Year of isolation	Species	Car-bapene-mase(s)	MIC values (mg/L)[a] for:			KPC&MBL&OXA-48 disc tests[b]						O.K.N.V.I. RESIST-5 results
				MEM	ETP	IPM	TMO	MRP	MR+CL	MR+BO	MR+ED	Interpreta-tion	
KP3648 (7)	2022	*K. pneumoniae*	NDM-5 + OXA-232	128	>1	>8	6	8	8	11	14	MBL	NDM + OXA-48-like
KP146 (7)	2023	*K. pneumoniae*	NDM-5 + OXA-232	64	>1	8	6	6	6	9	13	MBL	NDM + OXA-48-like
KP448 (7)	2023	*K. pneumoniae*	NDM-5 + OXA-232	128	>1	>8	6	6	6	7	12	MBL	NDM + OXA-48-like
KP3161 (7)	2023	*K. pneumoniae*	NDM-5 + OXA-232	128	>1	>8	6	6	6	8	12	MBL	NDM + OXA-48-like
PM1421 (7)	2007	*P. mirabilis*	VIM-1	1	1	8	20	26	26	28	29	Negative	VIM
PM1502 (7)	2021	*P. mirabilis*	VIM-1	1	1	8	19	25	25	27	28	Negative	VIM
CF2748 (6)	2014	*C. freundii*	VIM-4	2	>1	4	6	22	22	24	27	MBL	VIM
SM502 (6)	2014	*S. marcescens*	VIM-4	2	>1	8	6	22	22	24	28	MBL	VIM
SM1281 (6)	2015	*S. marcescens*	VIM-4	16	>1	>8	8	18	18	20	26	MBL	VIM
SM666 (6)	2017	*S. marcescens*	VIM-4	32	>1	>8	6	15	15	18	26	MBL	VIM
SM681 (6)	2018	*S. marcescens*	VIM-4	64	>1	>8	6	9	9	12	23	MBL	VIM
SM791 (6)	2018	*S. marcescens*	VIM-4	16	>1	>8	6	17	17	20	27	MBL	VIM
SM2238 (6)	2018	*S. marcescens*	VIM-4	32	>1	>8	6	6	6	8	23	MBL	VIM
SM2704 (6)	2018	*S. marcescens*	VIM-4	128	>1	>8	6	6	6	9	23	MBL	VIM
SM3131 (6)	2018	*S. marcescens*	VIM-4	64	>1	>8	6	8	8	11	24	MBL	VIM
SM1524 (6)	2020	*S. marcescens*	VIM-4	32	>1	>8	6	10	10	12	22	MBL	VIM
SM2942 (6)	2020	*S. marcescens*	VIM-4	32	>1	>8	6	10	10	12	23	MBL	VIM
PS316 (6)	2017	*P. stuartii*	VIM-86	16	>1	>8	14	18	18	20	22	MBL	VIM
PS314 (6)	2019	*P. stuartii*	VIM-86	16	>1	>8	13	18	18	20	25	MBL	VIM
PS995 (6)	2019	*P. stuartii*	VIM-86	16	>1	>8	15	18	18	20	25	MBL	VIM
PS3722 (6)	2019	*P. stuartii*	VIM-86	16	>1	>8	15	14	14	17	22	MBL	VIM
PS2654 (6)	2020	*P. stuartii*	VIM-86	16	>1	>8	11	17	17	20	24	MBL	VIM
PS3347 (6)	2020	*P. stuartii*	VIM-86	16	>1	>8	13	17	17	20	24	MBL	VIM
PS567 (6)	2019	*P. stuartii*	VIM-86 + NDM-1	32	>1	>8	10	16	16	18	24	MBL	VIM + NDM
PS1396 (6)	2019	*P. stuartii*	VIM-86 + NDM-1	64	>1	>8	10	11	11	13	24	MBL	VIM + NDM

[a] MIC, Minimum inhibitory concentration; MEM, meropenem; ETP, ertapenem; IMP, imipenem

[b] mm sone diameters KPC&MBL&OXA-48 disc kit; TMO, temocillin; MRP, meropenem; MR+CL: meropenem+cloxacillin; MR+BO, meropenem+phenylboronic acid; MR+ED, meropenem+EDTA

Figure 1. Example of a positive test for KPC carbapenemase using O.K.N.V.I. RESIST-5: left, cassette for OXA-48-like, KPC and NDM; right, cassette for VIM and IMP. C, control band; O, OXA-48-like; K, KPC; N, NDM; V, VIM; I, IMP carbapenemases. **Antibiogram:** Positive combination disc test for KPC-producing *K. pneumoniae* KP746 using KPC&MBL&OXA-48 disc kit. Clockwise: MRP, meropenem; TMO, temocillin; MR+ED, meropenem with EDTA; MR+BO, meropenem with phenylboronic acid; MR+CL, meropenem with cloxacillin.

Figure 2. Example of a positive test for OXA-48-like carbapenemase using O.K.N.V.I. RESIST-5: left, cassette for OXA-48-like, KPC and NDM; right, cassette for VIM and IMP. C, control band; O, OXA-48-like; K, KPC; N, NDM; V, VIM; I, IMP carbapenemases. **Antibiogram:** Positive combination disc test for OXA-48-like-producing *S.marcescens* SM585 using KPC&MBL&OXA-48 disc kit. Clockwise: MRP, meropenem; TMO, temocillin; MR+ED, meropenem with EDTA; MR+BO, meropenem with phenylboronic acid; MR+CL, meropenem with cloxacillin.

Figure 3. Example of a positive test for NDM carbapenemase using O.K.N.V.I. RESIST-5: left, cassette for OXA-48-like, KPC and NDM; right, cassette for VIM and IMP. C, control band; O, OXA-48-like; K, KPC; N, NDM; V, VIM; I, IMP carbapenemases. **Antibiogram:** Positive combination disc test for NDM-producing *K. pneumoniae* KP740 using KPC&MBL&OXA-48 disc kit. Clockwise: MRP, meropenem; TMO, temocillin; MR+ED, meropenem with EDTA; MR+BO, meropenem with phenylboronic acid; MR+CL, meropenem with cloxacillin.

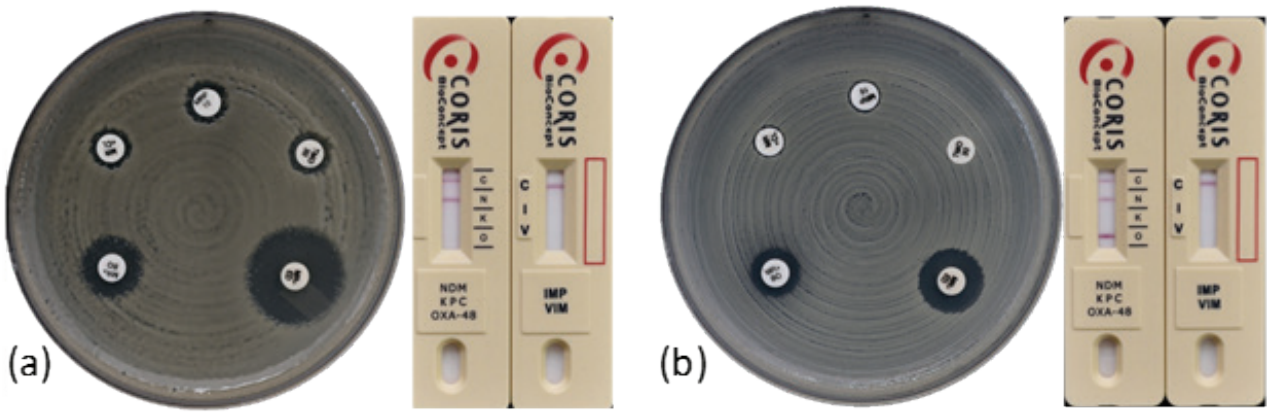

Figure 4. **(a)** Example of a positive test for NDM carbapenemase using O.K.N.V.I. RESIST-5: left, cassette for OXA-48-like, KPC and NDM; right, cassette for VIM and IMP. C, control band; O, OXA-48-like; K, KPC; N, NDM; V, VIM; I, IMP carbapenemases. **Antibiogram:** Positive combination disc test for NDM-producing *K. pneumoniae* KP166 using KPC&MBL&OXA-48 disc kit. Clockwise: MRP, meropenem; TMO, temocillin; MR+ED, meropenem with EDTA; MR+BO, meropenem with phenylboronic acid; MR+CL, meropenem with cloxacillin. **(b)** Example of a positive test for NDM and OXA-48-like carbapenemases using O.K.N.V.I. RESIST-5: left, cassette for OXA-48-like, KPC and NDM; right, cassette for VIM and IMP. C, control band; O, OXA-48-like; K, KPC; N, NDM; V, VIM; I, IMP carbapenemases. **Antibiogram:** Positive combination disc test for NDM and OXA-48-like-producing *K. pneumoniae* KP146 using KPC&MBL&OXA-48 disc kit. Clockwise: MRP, meropenem; TMO, temocillin; MR+ED, meropenem with EDTA; MR+BO, meropenem with phenylboronic acid; MR+CL, meropenem with cloxacillin.

from *P. mirabilis* strains, which were not detected. Furthermore, no co-production of OXA-48-like was detected in four isolates of *K. pneumoniae*. These isolates, which carried NDM-5 and OXA-232 carbapenemases, were interpreted as MBL producers.

The results obtained with the O.K.N.V.I. RESIST-5 and KPC&MBL&0XA-48 disc tests are illustrated in

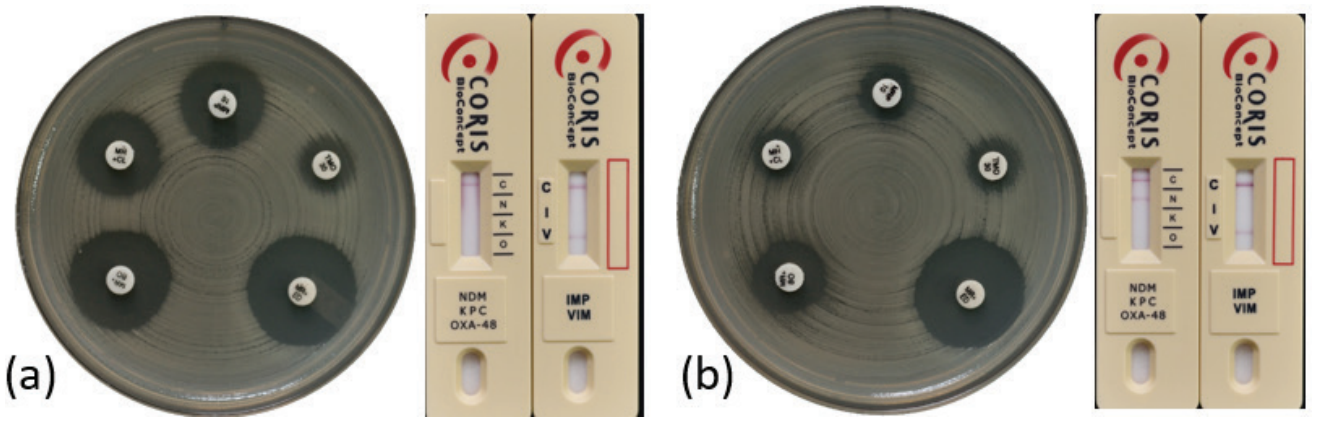

Figure 5. **(a)** Example of a positive test for VIM carbapenemase using O.K.N.V.I. RESIST-5: left, cassette for OXA-48-like, KPC and NDM; right, cassette for VIM and IMP. C, control band; O, OXA-48-like; K, KPC; N, NDM; V, VIM; I, IMP carbapenemases. **Antibiogram:** Positive combination disc test for VIM-producing *P. stuartii* PS2654 using KPC&MBL&OXA-48 disc kit. Clockwise: MRP, meropenem; TMO, temocillin; MR+ED, meropenem with EDTA; MR+BO, meropenem with phenylboronic acid; MR+CL, meropenem with cloxacillin. **(b)** Example of a positive test for NDM and VIM carbapenemases using O.K.N.V.I. RESIST-5: left, cassette for OXA-48-like, KPC and NDM; right, cassette for VIM and IMP. C, control band; O, OXA-48-like; K, KPC; N, NDM; V, VIM; I, IMP carbapenemases. **Antibiogram:** Positive combination disc test for NDM and VIM-producing *P. stuartii* PS1396 using KPC&MBL&OXA-48 disc kit. Clockwise: MRP, meropenem; TMO, temocillin; MR+ED, meropenem with EDTA; MR+BO, meropenem with phenylboronic acid; MR+CL, meropenem with cloxacillin.

comparative aspect in Figure 1 for KPC, in Figure 2 for OXA-48-like, in Figure 3 for NDM, in Figure 4 for NDM with and without OXA-48-like, and in Figure 5 for VIM with and without NDM.

DISCUSSION

The O.K.N.V.I. RESIST-5 multiplex immunochromatographic assay can detect the five most common carbapenemases. In a collection of 50 molecularly characterized clinical isolates, O.K.N.V.I. RESIST-5 performed excellently, detecting 7 carbapenemases produced by 8 species, with sensitivity and specificity of 100% for all OXA-48-like, KPC, NDM and VIM variants. Our results are consistent with data reported for previous versions of the assay when colonies were harvested around an ertapenem or meropenem disc on an MHA plate (4,5). It is notable that most false-negative results reported in the literature involved VIM or NDM producers that exhibited low carbapenem MIC levels (4,5,12). This could be related to the low expression of VIM or NDM enzymes, which can be overcome with the use of inoculum harvested around an ertapenem or meropenem disc, as antibiotic selection pressure leads to an increase in carbapenemase production. Based on these findings, Greissl et al. recommend the use of routine antibiogram to perform O.K.N.V.I. RESIST-5 if disk diffusion is the primary method of susceptibility testing, or the use of MHA or sheep blood agar with an ertapenem or meropenem disk for purity control when an automated susceptibility testing system is used. In such an approach, the results of the antibiogram and the identified carbapenemase are obtained simultaneously, which would help physicians to prescribe adequate therapy and appropriate infection control measures.

CONCLISION

Our results show that the O.K.N.V.I. RESIST-5 assay has excellent performance in detecting all carbapenemase genes present in the collection we studied. For best results, isolates should be harvested around an ertapenem or meropenem disc from a routine antibiogram. In addition, the O.K.N.V.I. RESIST-5 is a rapid, simple and efficient tool for implementation in the clinical microbiology laboratory.

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