Original Article

Carbapenemase detection by NG-Test CARBA 5—a rapid immunochromatographic assay in carbapenem-resistant *Enterobacterales* diagnosis

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Background: The global spread of carbapenem-resistant *Enterobacterales* (CRE) represents a serious public health concern as these organisms are associated with limited treatment options, high mortality rate and rapid transmissibility. The identification of carbapenemase remains a challenge in microbiological laboratories as no single method is perfect when considering cost, carbapenemase coverage, accuracy, handling complexity and TATs together.

Methods: NG-Test CARBA 5 assay and modified carbapenem inactivation method in conjunction with EDTA carbapenem inactivation method (mCIM/eCIM) were challenged with a collection of 299 molecularly characterized CRE isolates in China in order to evaluate the performance in detecting five major carbapenemases (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48}) among Enterobacterales.

Results: NG-Test CARBA 5 detected all KPC-, NDM-, VIM- and OXA-48-producing isolates perfectly with a weak false-positive signal for NDM in an IMP-4 producer, which makes the specificity for NDM decreases to 99.6%. The overall specificity/sensitivity were 99.9%/100% for NG-Test CARBA 5. mCIM/eCIM achieved high specificity of 100%/100% and sensitivity of 99.6%/97.4%, with one S. marcescens isolate harboring VIM-2 undetected.

Conclusions: Both NG-Test CARBA 5 and mCIM/eCIM showed excellent results in the tested carbapenemase (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48}) detection compared with molecular genotypic test. As every assay has its own limitations, suitable methods should be combined for the establishment of the CRE diagnostic pathways.

Keywords: Carbapenemase-resistant *Enterobacterales* (CRE); EDTA carbapenem inactivation method (eCIM); modified carbapenem inactivation method (mCIM); NG-Test CARBA 5

Submitted Dec 29, 2020. Accepted for publication Mar 05, 2021.
doi: 10.21037/atm-20-8216
View this article at: http://dx.doi.org/10.21037/atm-20-8216

Introduction

The global spread of carbapenem-resistant *Enterobacterales* (CRE) represents a serious public health concern as these pathogens are associated with limited treatment options, high mortality rate and rapid transmissibility (1,2). Mechanisms underlying carbapenem resistance are related mainly to the production of carbapenemases (carbapenemase-producing *Enterobacterales*, CP-CRE), but also a consequence of decreased membrane permeability coupled with the overexpression of extended-spectrum β-lactamases (ESBLs) or AmpC β-lactamases (non-carbapenemase-producing carbapenem-resistant *Enterobacterales*, non-CP-CRE) (3-5). Detection of the mechanisms of carbapenem resistance is not routinely performed in many Chinese hospitals. However, the distinction between CP-CRE and non-CP-CRE is valuable for infection control as CP-CRE can spread rapidly through horizontal gene transfer among Gram-negative organisms and therefore may require implementation of intensive infection control interventions (3,5). Moreover, novel antimicrobial agents possess unique profiles in their activities against different carbapenemases. Ceftazidime-avibactam is active on organisms harboring serine-carbapenemase, but shows no effects on metallo-β-lactamase (MBL) producers (6-8). Meropenem-vaborbactam has activity against KPC-producing pathogens, while it remains ineffective to OXA-48-like and MBL-producing *Enterobacterales* (9). Therefore, the identification of carbapenemases is important for making effective treatment decisions.

A variety of phenotypic and genotypic tests can be chosen for CP-CRE detection (10). Modified carbapenem inactivation method in conjunction with EDTA carbapenem inactivation method (mCIM/eCIM), recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines, yields high accuracy in carbapenemase detecting, but has long turn-around times (TATs) (3). Molecular genetic approach is regarded as the reference standard and allows the rapid identification of carbapenemase genes either in cultured bacteria or directly from clinical samples (5). However, its performance can be limited by the presence of new variants or rare enzymes which are not detectable by molecular tests (2,11). Furthermore, the expensive instrument and high cost of its reagent restrict the widely application of molecular tests, including GeneXpert (12,13). A newly developed lateral flow immunoassay, NG-Test CARBA 5 (NG-Biotech, France), can detect the five most prevalent carbapenemases of KPC, NDM, IMP, VIM and OXA-48-like within 15 minutes. High sensitivity (from 97.3% to 100%) and specificity (from 96.1% to 100%) of NG-Test CARBA 5 have been reported from studies conducted mainly in Europe (14-19). With simple experimental procedures and short TATs, NG-Test CARBA 5 is a promising method for the CPE detection.

The aim of this study was to compare the performance of NG-Test CARBA 5 and mCIM/eCIM in detecting five major carbapenemases among *Enterobacterales* with molecular method.

We present the following article in accordance with the STARD reporting checklist (available at http://dx.doi.org/10.21037/atm-20-8216).

Methods

Bacterial isolates

A total of 301 isolates were employed in this study, comprising 299 clinical CRE isolates and two quality control (QC) isolates. The 299 clinical CRE isolates, which were collected from respiratory, sterile fluids, tissues, blood, rectal swab/stool, urine, etc., were referred by 20 hospitals in China, from 2007 to 2018, to Peking Union Medical College Hospital (PUMCH). All the *Enterobacterales* isolates were identified by MALDI-TOF MS (Bruker Biotyper; Bruker Daltonik, Bremen, Germany). CRE was defined as non-susceptible to imipenem, non-susceptible to meropenem or resistant to ertapenem, by the broth microdilution method as per breakpoints criteria of CLSI M100-S30 (20). Isolates recovered from frozen stock (–80 ℃) were subcultured twice on blood agar plate with a meropenem disk placed and incubated at 35±2 ℃ overnight to ensure purity.

Molecular detection of carbapenemase genes

bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48} genes were detected by multiplex PCR according to Poirel *et al.* (21). Non-CP-CRE were further analyzed for the presence of plasmid-mediated AmpC β-lactamase or ESBL genes bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{CMY} and bla_{DHA} (22,23). All positive isolates were sent for subsequent DNA sequencing and the obtained gene sequences were compared with NCBI nucleotide database using blast server (http://blast.ncbi.nlm.nih.gov). Sequences were considered a match if 100% sequence identity of coding sequence (CDS) and 100% coverage were present (11).

NG-Test CARBA 5

The tests were performed as per manufacturer's instructions. Briefly, a 1 µL loopful of bacteria was mixed with five drops of extraction buffer, and 100 µL of the mixture was dispensed into the CARBA-5 cassette. Results were interpreted after 15 min of incubation at room temperature (3).

mCIM/eCIM

The tests were performed and interpreted as per instructions from CLSI M100-S30 (20). *Klebsiella pneumoniae* ATCC BAA-1706 and ATCC BAA-1705 were used as negative and positive quality controls, respectively.

Statistical analysis

The investigators performing NG-Test CARBA 5 and mCIM/eCIM were blind to the identities of the isolates. Sensitivity and specificity were calculated with a 95% confidence interval (CI) based on an exact binomial distribution. SPSS, version 15.0 (SPSS Inc., Chicago, USA) was used for data analysis.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by The Human Research Ethics Committee of Peking Union Medical College Hospital of S-K1056 and informed consent was waived as the study collected remaining samples from clinical tested samples without extra operation of patients.

Results

Distribution of species and carbapenemases

Eight species were identified in 299 CRE isolates, including 175 *Klebsiella pneumoniae* (58.5%), 48 *Escherichia coli* (16.1%), 46 *Enterobacter cloacae* (15.4%), 9 *Klebsiella oxytoca* (3.0%), 8 *Klebsiella aerogenes* (2.7%), 8 *Citrobacter freundii* (2.7%), 4 *Serratia marcescens* (1.3%), and 1 *Proteus mirabilis* (0.3%). A total of 253 isolates were positive for carbapenemase genes, among which 172 KPC (68.0%), 29 NDM (11.5%), 41 IMP (16.2%), 3 OXA-48 (1.2%), 4 VIM (1.6%), 2 IMP and VIM co-existing (0.8%), as well as 2 KPC and IMP co-existing (0.8%) genes were identified (*Table 1*).

Table 1 Overview of the 299 clinical CRE isolates tested for the evaluation of NG-Test CARBA 5 and mCIM/eCIM

Carbapenemase	*Klebsiella pneumoniae*	*Escherichia coli*	*Klebsiella aerogenes*	*Enterobacter cloacae*	*Klebsiella oxytoca*	*Citrobacter freundii*	*Proteus mirabilis*	*Serratia marcescens*	Total
CP-CRE (n=253)									
KPC (n=172)									
KPC-2	132	24	4	5	1		1	3	170
KPC-3	1								1
KPC-4	1								1
NDM (n=29)									
NDM-1	6	4		15	1	1			27
NDM-4		1							1
NDM-5		1							1
IMP (n=41)									
IMP-1	1	2		2	1	4			10
IMP-4	7		1	10	4	2			24
IMP-8	4			1					5
IMP-26	1			1					2

Table 1 (*continued*)

Table 1 (*continued*)

Carbapenemase	*Klebsiella pneumoniae*	*Escherichia coli*	*Klebsiella aerogenes*	*Enterobacter cloacae*	*Klebsiella oxytoca*	*Citrobacter freundii*	*Proteous mirabilis*	*Serratia marcescens*	Total
OXA (n=3)									
OXA-48	1	2							3
VIM (n=4)									
VIM-1	1								1
VIM-2								1	1
VIM-4		1							1
VIM-19	1								1
Double carbapenemases (n=4)									
KPC-2 + IMP-4				1	1				2
IMP-1 + VIM-1				2					2
Non-CP-CRE (n=46)									
AmpC β-lactamase (n=3)									
CMY		1				1			2
DHA			1						1
ESBL (n=20)									
CTX-M	1	3	1	2					7
CTX-M + TEM		4		3					7
SHV + CTX-M	4								4
CTX-M + TEM + SHV	2								2
AmpC β-lactamase + ESBL (n=23)									
DHA + CTX-M			1	2					3
TEM + DHA				1					1
TEM + CMY		1							1
SHV + DHA	2								2
TEM + CTX-M + DHA				1					1
TEM + CTX-M + CMY		3							3
SHV + CTX-M + DHA	5				1				6
SHV + CTX-M + CMY	1								1
TEM + SHV + DHA	1								1
TEM + SHV + CMY	1								1
TEM + SHV + CTX-M + DHA	2	1							3
Total	175	48	8	46	9	8	1	4	299

mCIM, modified carbapenem inactivation method in conjunction; eCIM, EDTA carbapenem inactivation method; CRE, carbapenem-resistant *Enterobacterales*; IMP, imipenemase; VIM, Verona integron-encoded metallo-beta-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillin-hydrolyzing class D beta-lactamase; ESBL, extended-spectrum β-lactamases; CTX-M, cefotaximase; TEM, temoneira; SHV, sulfhydryl variable; DHA, Dhahran Hospital in Saudi Arabia; CMY, cephamycinase.

Table 2 Comparison of the performance of NG-Test CARBA 5 versus mCIM/eCIM for carbapenemase detection using 299 clinical CRE isolates

	NG-Test CARBA 5		mCIM		eCIM	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
All isolates	100 (98.1–100)	99.9 (99.5–100)	99.6 (97.5–100)	100 (90.4–100)	97.4 (90.1–99.5)	100 (97.3–100)
KPC	100 (97.3–100)	100 (96.3–100)	100 (97.3–100)	100 (90.4–100)	NA	100 (97.3–100)
NDM	100 (85.4–100)	99.6 (97.6–100)	100 (85.4–100)	100 (90.4–100)	100 (85.4–100)	100 (97.3–100)
IMP	100 (89.3–100)	100 (98.2–100)	100 (89.3–100)	100 (90.4–100)	100 (89.3–100)	100 (97.3–100)
OXA-48	100 (30.1–100)	100 (98.4–100)	100 (31.0–100)	100 (90.4–100)	NA	100 (31.0–100)
VIM	100 (39.6–100)	100 (98.4–100)	75 (21.9–98.7)	100 (90.4–100)	100 (31.0–100)	100 (97.3–100)
KPC+IMP	100 (19.8–100)	100 (98.4–100)	100 (19.8–100)	100 (90.4–100)	0 (0–80.2)	100 (97.3–100)
VIM+IMP	100 (19.8–100)	100 (98.4–100)	100 (19.8–100)	100 (90.4–100)	100 (19.8–100)	100 (97.3–100)
K. pneumoniae	100 (97.0–100)	100 (99.3–100)	100 (97.0–100)	100 (79.1–100)	100 (80.8–100)	100 (91.6–100)
E. coli	100 (87.7–100)	100 (97.7–100)	100 (87.7–100)	100 (71.7–100)	100 (62.9–100)	100 (84.0–100)
E. cloacae	100 (89.1–100)	99.5 (96.7–100)	100 (88.3–100)	100 (62.9–100)	96.9 (82.0–99.8)	100 (46.3–100)
K. oxytoca	100 (62.9–100)	100 (88.0–100)	100 (59.8–100)	100 (5.5–100)	85.7 (42.0–99.2)	100 (54.6–100)
K. aerogenes	100 (46.3–100)	100 (87.7–100)	100 (46.3–100)	100 (31.0–100)	100 (5.5–100)	100 (39.6–100)
C. freundii	100 (56.1–100)	100 (87.0–100)	100 (56.1–100)	100 (5.5–100)	100 (56.1–100)	NA
S. marcescens	100 (39.6–100)	NA	75 (21.9–98.7)	NA	NA	100 (31.0–100)
P. mirabilis	100 (5.5–100)	NA	100 (5.5–100)	NA	NA	100 (5.5–100)

The numbers in bracket indicate 95% confidence interval. mCIM, modified carbapenem inactivation method in conjunction; eCIM, EDTA carbapenem inactivation method; CRE, carbapenem-resistant *Enterobacterales*; NA, not available.

Performances of NG-Test CARBA 5 and eCIM/mCIM in comparison with genotypic test

Both NG-Test CARBA 5 and mCIM/eCIM showed excellent results in the carbapenemase detection compared with genotypic test. The performance of the two tests is illustrated in *Table 2* and discrepant results are listed in *Table 3*. When calculating analytical specificity and sensitivity, each NG-Test CARBA 5 assay was considered as five individual tests, meaning a total of 1,495 (299×5) tests were conducted. The overall specificity and sensitivity of NG-Test CARBA 5 were 99.9% and 100%, respectively. NG-Test CARBA 5 detected all KPC-, NDM-, VIM- and OXA-48-producing isolates perfectly. However, an IMP-4 producer gave an additional weak false-positive signal for NDM, which makes the specificity for NDM decrease to 99.6%. mCIM/eCIM achieved high specificity of 100%/100% and sensitivity of 99.6%/97.4%, with one *S. marcescens* isolate harboring VIM-2 undetected. Additionally, two isolates genetically characterized as carrying both bla_{KPC} and bla_{IMP} yielded mCIM-positive (zone diameter =6 mm) but eCIM-negative results (zone diameter = 6mm), which failed to report the presence of MBL. It is expected as EDTA does not inhibit KPC. Five IMP producers, which possessed relatively low level of carbapenem resistance compared with other isolates, were correctly identified by NG-Test CARBA 5 and mCIM/eCIM (*Table 4*). As for non-CP-CRE isolates harboring either ESBLs or AmpC β-lactamase, both tests yielded negative results. When it came to the performances of the two assays on different isolates, *K. pneumoniae*, *E. coli*, *K. aerogenes*, *C. freundii* and *P. mirabilis* were all correctly identified. NG-Test CARBA 5 failed in an *E.cloacae* isolate and *mCIM/eCIM* failed in *S. marcescens*, *E. cloacae* and *K. oxytoca isolates* (*Table 3*).

Discussion

Rapid and accurate detection of CP-CRE is of great necessity for both treatment decision making and implementation of infection control. The main features of NG-Test CARBA5, mCIM/eCIM and genotypic test are

Table 3 Characteristics of isolates showing discrepant phenotypic results with genotypic results

Organism	Carbapenemase	NG-Test CARBA5 result	mCIM result	eCIM result	MIC (μg/mL)		
					Imipenem	Meropenem	Ertapenem
Enterobacter cloacae	IMP-4	IMP+NDM	+	+	8 (R)	16 (R)	64 (R)
Serratia marcescens	VIM-2	VIM	–	–	>256 (R)	>256 (R)	>256 (R)
Enterobacter cloacae	KPC-2+IMP-4	KPC+IMP	+	–	16 (R)	64 (R)	16 (R)
Klebsiella oxytoca	KPC-2+IMP-4	KPC+IMP	+	–	32 (R)	32 (R)	64 (R)

mCIM, modified carbapenem inactivation method in conjunction; eCIM, EDTA carbapenem inactivation method; IMP, imipenemase; VIM, Verona integron-encoded metallo-beta-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; R, resistant.

Table 4 Results of NG-Test CARBA 5 and mCIM/eCIM for the detection of CRE with relatively low level of carbapenem resistance

Organism	Carbapenemase	NG-Test CARBA 5 result	mCIM/eCIM result	MIC (μg/mL)		
				Imipenem	Meropenem	Ertapenem
Klebsiella oxytoca	IMP-1	+	+	2 (I)	2 (I)	2 (R)
Klebsiella oxytoca	IMP-4	+	+	1 (S)	1 (S)	2 (R)
Klebsiella oxytoca	IMP-4	+	+	2 (I)	4 (R)	2 (R)
Klebsiella pneumoniae	IMP-4	+	+	2 (I)	4 (R)	2 (R)
Klebsiella pneumoniae	IMP-4	+	+	2 (I)	4 (R)	2 (R)

mCIM, modified carbapenem inactivation method in conjunction; eCIM, EDTA carbapenem inactivation method; CRE, carbapenem-resistant *Enterobacterales*; IMP, imipenemase; MIC, minimum inhibitory concentration; I, intermediate; S, susceptible; R, resistant.

Table 5 Comparison of the main features of three carbapenemase detection methods

Parameter	NG-Test CARBA 5	mCIM/eCIM	Xpert® CARBA R
Sample type	Bacterial colonies, blood cultures	Bacterial colonies	Bacterial colonies, rectal swabs
Cost	Moderate	Low	High
Special instruments required	No	No	Yes
Experiment simplicity	Simple	Moderate	Simple
Time-to-result	15 min	18–24 h	1 h
Result interpretation	Simple	Moderate	Simple
Cover range for carbapenemase	KPC, NDM, VIM, IMP, OXA-48-like	Serine carbapenemase, MBL	KPC, NDM, VIM, IMP, OXA-48-like

mCIM, modified carbapenem inactivation method in conjunction; eCIM, EDTA carbapenem inactivation method; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; VIM, Verona integron-encoded metallo-beta-lactamase; IMP, imipenemase; OXA, oxacillin-hydrolyzing class D beta-lactamase.

illustrated in *Table 5*. As every assay has its own limitations, two phenotypic assays were compared with genotypic test to choose suitable methods for the establishment of the CRE diagnostic pathways.

The CLSI guideline stated that mCIM demonstrated >99% sensitivity and specificity, while eCIM showed >95% sensitivity and >92% specificity when detecting the five major types of carbapenemases among *Enterobacterales* isolates (20). Our results coincided with it, with sensitivity of mCIM and eCIM being 99.6% and 97.4%, respectively, and specificity both being 100%. mCIM/eCIM is able to identify rare carbapenemases like GES-4, IMP-6- and NDM-1, which are beyond the detection range of NG-Test CARBA 5 (24). Additionally, result interpreting of

mCIM/eCIM is objective and convenient as it depends on a defined zone diameter. The majority of the isolates tested here exhibited a mCIM zone diameter of 6 mm and eCIM zone diameter of approximately 15 mm, which confirmed its ease of interpretation. One limitation of mCIM/eCIM is its inability to differentiate between MBLs and serine carbapenemases when the organism harbor both of them (3,7). The number of organisms encoding double carbapenemases is enlarging, which is confirmed by an observation that the annual incidence of KPC-2/NDM-1 *K. pneumoniae* is increasing. The rate was zero from 2012 to 2015 and rose to 0.28% in 2016, finally reaching 0.58% in 2017 (25). Due to the higher frequency of double-enzyme producers, methods that can correctly identify between MBL and serine carbapenemases are of great need, as organism producing different enzymes leads to diverse therapies. Another limitation of mCIM/eCIM is it requires an overnight incubation which prolongs TATs.

NG-Test CARBA 5 is a newly developed assay for the carbapenemase detection. In this study, NG-Test CARBA 5 achieved high sensitivity of 100%. Organisms co-producing serine cabapenemase and MBL were precisely differentiated, which is especially valuable as mCIM/eCIM cannot identify them. The specificity of NG-Test CARBA 5 decreased to 99.9% due to an additional NDM false-positive band from an IMP-4 *E. cloacae*. False-positive results were rarely reported, with the exception of two wrong signals for VIM and KPC bands observed in another study evaluating 197 isolates (15). KPC and NDM are the two carbapenemases most frequently identified in Chinese hospitals (26-28). NG-Test CARBA 5 exhibited good performance with no false-negative but a false-positive result in their detection. NG-Test CARBA 5 showed advantages in the identification of OXA-48-like and IMP. The detection of OXA-48-like by phenotypic methods can be challenging as the hydrolysis activity of OXA-48-like is generally weak (29). NG-Test CARBA 5, based on the recognition of conserved epitopes of the carbapenemase, is not influenced by it and therefore has advantages in the detection for OXA-48-like. Except for OXA-48, NG-Test CARBA 5 has also correctly identified several OXA-48 variants, e.g., OXA-181, OXA-204, and OXA-162 (19). However, as it has been reported, the high sequence diversity within IMP family makes it more difficult to be detected (15). In this study, Organisms producing IMP-1, IMP-4, IMP-8, and IMP-26 were all correctly identified. With wide coverage for IMP variants, NG-Test CARBA 5 is a great tool for carbapenemase detection in areas where IMP is prevalent. One disadvantage for NG-Test CARBA 5 is that despite being able to identify nearly all variants of NDM, KPC, VIM and OXA-48-like, rare carbapenemases are out of its detection range (17). For example, GIM, SME, IMI are undetectable by NG-Test CARBA 5 (17,19).

CRE infection, especially CRE blood stream infection, is associated with high mortality. In a study reviewing bloodstream infection cases, patients with CRE bacteremia had a median of 52-hour from culture collection until receipt of active therapy and the 52-hour delay was associated with increased mortality. Additionally, 21% of patients with CRE bacteremia did not receive active therapy as they died prior to the availability of antimicrobial susceptibility testing reporting (30). Therefore, rapid diagnosis of CRE is essential for early treatment of patients. As for the current CRE diagnostic pathway in PUMCH, antimicrobial susceptibility testing is performed firstly after the bacterial identification, and then mCIM/eCIM is conducted to CRE isolate for the confirmation of its carbapenem-resistant mechanisms. It takes approximately 48h from sample reception to the reporting of antimicrobial susceptibility testing and results for carbapenemase testing require an additional night.

A major limitation of this study is that cabapenemases that could not be detected by NG-Test CARBA 5 were not fully covered. In addition, this study focused only on *Enterobacterales*. However, non-fermenters like *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are also frequently encountered organisms which produce carbapenemase in the clinical practice. The performance of NG-Test CARBA 5 and mCIM/eCIM on these organisms should be further investigated. What's more, the diversity of carbapenemases tested in this study is limited. Only 4 VIM-producing isolates and 3 OXA-producing isolates were included, while 172/253 were KPC producers. More VIM and OXA isolates should be tested in further study.

Acknowledgments

Funding: This study was supported by National Natural Science Foundation of China (82072318), National Key Research and Development Program of China (2018YFE0101800, 2018YFC1200105), Beijing Key Clinical Specialty for Laboratory Medicine - Excellent Project (No. ZK201000). The funders were not involved in the study design, collection, analysis, interpretation of data, writing of this article, or the decision to submit it for publication.

Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at http://dx.doi.org/10.21037/atm-20-8216

Data Sharing Statement: Available at http://dx.doi.org/10.21037/atm-20-8216

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-8216). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by The Human Research Ethics Committee of Peking Union Medical College Hospital of S-K1056 and informed consent was waived as the study collected remaining samples from clinical tested samples without extra operation of patients.

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Cite this article as: Zhu Y, Jia P, Li X, Wang T, Zhang J, Zhang G, Duan S, Kang W, Xu Y, Yang Q. Carbapenemase detection by NG-Test CARBA 5—a rapid immunochromatographic assay in carbapenem-resistant *Enterobacterales* diagnosis. Ann Transl Med 2021;9(9):769. doi: 10.21037/atm-20-8216

