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Evaluation of NG-Test CARBA 5 with Carbapenemase Producing Organisms and Non-Carbapenemase Producing Organisms

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Revised Abstract

Background: According to the CDC, carbapenem-resistant *Enterobacteriaceae* (CRE) in the United States is considered an urgent threat. Identification of carbapenemase genes and decreased susceptibility to carbapenems relies mostly on expensive molecular techniques, Kirby Bauer disk diffusion, or alternative screening culture media. NG-Test CARBA 5 is an *in vitro* rapid and visual multiplex immunoassay for the qualitative detection of 5 common carbapenemases from bacterial colonies. This test is easy to use and interpret, requires little hands on time (1 minute), is low cost, and identifies a positive phenotype and the type of carbapenemase being produced at the same time. The implementation of this test can help prevent the spread of these organisms as well as improve treatment of carbapenem resistant organisms (CROs) as some antimicrobial agents are only effective against serine or metallo-beta lactamases, but not both.

Methods: In order to determine the analytical sensitivity of NG-Test CARBA 5, ninety-one clinical strains known for harboring at least one of five different carbapenemases (KPC, OXA-48-like, VIM, IMP, and NDM) were tested with the NG-Test CARBA 5 kit in triplicate from 5% sheep's blood and MacConkey agar. In order to determine the analytical specificity of NG-Test CARBA 5, eighty-one organisms that exhibit antibiotic resistance mechanisms other than the lateral flow assay targets, such as AmpC, IMI, SME, and GES, are carbapenem-susceptible, or are carbapenem non-susceptible were also tested on NG-Test CARBA 5 from blood agar and MacConkey agar. Organisms tested included *Enterobacteriaceae* and *Pseudomonas aeruginosa*, as well as other phylogenetically related organisms. Additionally, forty clinical *Enterobacteriaceae* strains were tested with the kit in triplicate on the recently FDA cleared HardyCHROM™ CRE (HC CRE) agar. Fifteen non-target *Enterobacteriaceae* were also tested from HC CRE agar. The test reader was blinded to the expected molecular characterization data and antibiotic susceptibility information while preparing each test and reading the test result.

Results: The sensitivity was 87/91 (95.6%) from blood agar and 89/91 (97.8%) from MacConkey agar. Two *Proteus mirabilis* showed a false negative CARBA 5 result from blood agar only; this may have been a result of the swarming morphology on blood agar. However, this type of result was not reproducible across all of the *P. mirabilis* tested from blood agar. All 81 non-target organisms that were tested from blood and MacConkey agar for specificity delivered 100% negative test results as expected. The sensitivity was 39/40 (98.2%) from HC CRE and all fifteen organisms with non-target resistance mechanisms showed a negative result on NG-Test CARBA 5. One IMP-14 producer (*Enterobacter asburiae*) was a false negative from all agars tested, and an additional IMP (variant not yet characterized) produced by *P. aeruginosa* was also a false negative from blood and MacConkey agar.

Conclusions: While molecular methodologies are known to be highly reliable and sensitive, a positive PCR result may require phenotypic confirmation to assess the expression of the gene. This evaluation showed that the NG-Test CARBA 5 reliably identifies the "Big 5" carbapenemases at a low cost and minimal turnaround time in order to improve patient treatments and prevent global AMR.

Introduction

Carbapenemase detection has become increasingly important in recent years due to the limited number of antimicrobial agents available to treat infections caused by carbapenemase producing organisms (CPO). The importance of identifying patients colonized with carbapenemase producing organisms has also increased since carbapenemases can spread within hospitals and in the community on plasmids. Interestingly, the breakpoints determined by CLSI and EUCAST encompass a majority of the carbapenemase producing organisms, but not all. Thus, hospitals must be vigilant in identifying suspicious cases and antibiograms with elevated MIC to carbapenems, even if it falls within the susceptible breakpoint. Although new antimicrobials have been and are currently being developed to target these organisms, some compounds only treat subgroups of CPO depending on the specific enzyme produced. Determining the enzyme responsible for resistance is no longer solely for epidemiological purposes; now it is more directly linked to patient care and infection control.

The NG-Test CARBA 5 lateral flow immunoassay is produced by immobilizing mouse monoclonal antibodies directed against KPC (K), OXA-48-like (O), VIM (V), IMP (I), and NDM (N) on the nitrocellulose membrane test zones K, O, V, I and N. The assay is carried out by dispensing the sample in the cassette well. The sample migrates towards the conjugate pad and, if present, the carbapenemase(s) react with labelled anti-carbapenemase monoclonal antibodies. The complex migrates through the nitrocellulose membrane via capillary action and interacts with the corresponding anti-carbapenemase monoclonal antibodies immobilized on the membrane. If the sample is positive for one or several carbapenemases, a red line will appear on the test zone(s) and on the control zone of the membrane. If not, only one red line will appear on the control zone.

Methods

Bacterial strains from Hardy Diagnostics' bacterial isolate collection were inoculated from a frozen stock vial to an agar plate and were incubated aerobically at 35°C overnight. Each culture was transferred to a secondary agar plate the following day. After overnight incubation, the blood and MacConkey agar secondary cultures were used with NG-Test CARBA 5. For HC CRE, serial dilutions to $3x10^5$ CFU/mL were prepared from 18-24 hour cultures and then spiked into CRE-negative stool specimen in a 1:10 ratio. After vortexing, the spiked stool sample was streaked to HC CRE for isolated colonies. After blood and MacConkey secondary cultures had incubated for 16 hours and HC CRE had incubated for 18 hours, each media was tested by using a 1µL loop to touch three colonies before inoculating into the CARBA 5 extraction buffer. The NG-Test CARBA 5 instructions were followed for the rest of the test procedure. Bacterial isolates that were expected to produce a target carbapenemase were tested in triplicate with NG-Test CARBA 5 from each agar type. The operator was blinded to characterization results when testing each organism. Modified carbapenem inactivation method (mCIM, CLSI M100, S29) was performed in instances where there was a positive molecular characterization but a negative NG-Test CARBA 5 result (potential False Negative). NG-Test CARBA 5 devices were provided by NG Biotech, Guipry, France, for this evaluation.

Table 1. Analytical Reactivity Summary

Organism Group	Number of **strains** tested on Blood and MacConkey agar	Number of **strains** tested on HC CRE agar[4]	Number of **analytes** tested on Blood and MacConkey agar[2]	Number of **analytes** Tested HC CRE[2]	Analyte	Variants Tested	Variants Not Detected
Enterobacteriacae	66	40	17	8	KPC	2, 3, 4, 6*, 12	
			12	7	OXA-48-like	48, 181, 163, 232 (48 type)	
			11	9	VIM	1, 4, 5*, 6, 23, 27, 31	
			8	7	IMP	4, 8[3], 26*, 1, 47*	14
			15	11	NDM	1[1], 5, 6, 7	
			4	1	Negative		
Pseudomonas aeruginosa	25		5		KPC	2, 5	
			0		OXA-48-like		
			13		VIM	2, 11	
			5		IMP	1, 7, 19, 26	One undetermined
			2		NDM	1	
			0		Negative		

[1] NDM-1 and IMP-26 not detected in *P. mirabilis* growth from Blood agar, but yielded the expected NG-Test CARBA 5 result from MacConkey agar.
[2] The number of analytes tested and organisms do not match because some organisms produced multiple target carbapenemases.
[3] NG-Test CARBA 5 detects IMP-8 variant which has been identified as a False Negative by Xpert Carba-R. However, IMP-14 is still troublesome to detect among the IMP variants.
[4] HardyCHROM™ CRE is intended for detection of *Escherichia coli* and KES (*Klebsiella aerogenes*, *K. oxytoca*, *K. pneumoniae*, *Enterobacter cloacae* complex, and *Serratia marcescens*)
*Not tested on HardyCHROM™ CRE agar, only tested on blood and MacConkey agar.

Table 2. Number of Target and Non-Target *Enterobacteriaceae* Species Tested on NG-Test CARBA 5

Genus species	Number of organisms Tested from Blood/ MacConkey agar	Number of organisms Tested from HC CRE
Citrobacter freundii	3	
Citrobacter koseri	3	
Citrobacter species	1	
Enterobacter asburiae	3	3
Enterobacter cloacae	15	14
Enterobacter cloacae complex	2	2
Escherichia coli	24	10
Klebsiella aerogenes	2	3
Klebsiella oxytoca	7	3
Klebsiella ozaenae	1	
Klebsiella pneumoniae	27	16
Kluyvera ascorbata	1	
Morganella morganii	2	
Proteus mirabilis	12	
Providencia alcalifaciens	1	
Providencia stuartii	1	
Raoultella ornithinolytica	1	
Salmonella Senftenberg	1	
Serratia marcescens	10	4
Shigella boydii	1	
Shigella sonnei	1	
Yersinia enterocolitica	1	
Total	120	55

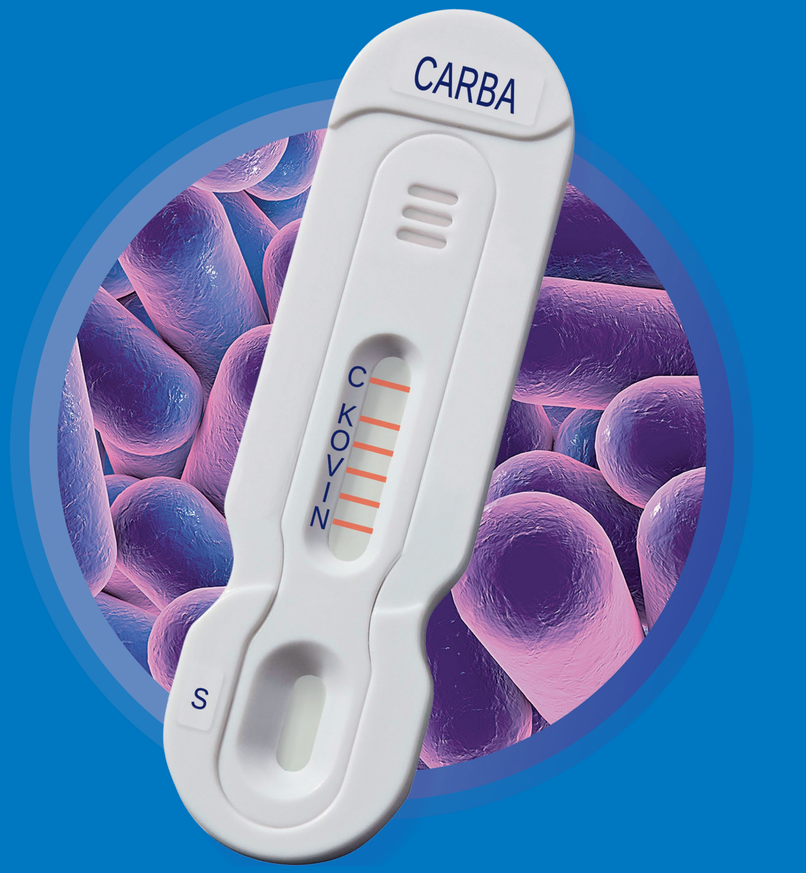
Table 3. Resistance Mechanisms Evaluated with NG-Test CARBA 5 for Specificity

AmpC	DHA-1	MIR-8
tet(A), tet(B), tet(C)	ACT-2	PER-1
CTX-M (14, 15, 30, 75, 79, 55, 8, 9, 22, 124, 40, 74, 1, 3, 24)	OXA (30, 1, 50, 10)	IMI
SHV (31, 108(u), 12(2be), 179(u), 11(2b), 180(u), 182(u), 89(2b), -OSBL(2b), 154, 28) (sulfhydryl variable)	PAO	mcr-1, ESBL
TEM (1, 1(2be), 11(2be), -OSBL(2b), 210(u), 63(2be), 93(2be))	PDC (1, 5, 35, 19)	VEB-1
catB7	aph(3')-IIb, aadB, aadA6	GES (1, 5(c))
SME-2	strA, strB	sul1

Results

87/91 (95.6%) of target organisms tested indicated the expected result on NG-Test CARBA 5 from blood agar and 89/91 (97.8%) of target organisms tested indicated the expected result on NG-Test CARBA 5 from MacConkey Agar. A subset of the same organisms tested from blood and MacConkey agar were also tested from HC CRE agar. 39/40 (97.5%) of the target *Enterobacteriaceae* tested from HC CRE agar delivered the expected results on NG-Test CARBA 5. **Table 1** shows the total number of target analytes tested with NG-Test CARBA 5 from each agar type as well as the specific variants evaluated (if known). One IMP-14 producer (*Enterobacter asburiae*) was a false negative on all agar types tested and an unknown IMP variant (*Pseudomonas aeruginosa*) was a false negative on blood and MacConkey agar. For two *Proteus mirabilis* strains, the test result was negative from blood agar and positive from MacConkey agar. For these two particular strains, swarming was observed on blood agar but not on MacConkey agar. This was the only difference observed in performance between blood and MacConkey agar with NG-Test CARBA 5.

Table 2 indicates the number of each *Enterobacteriaceae* species evaluated from each agar type in analytical and cross reactivity testing combined. Overall, 45 strains of *P. aeruginosa* were evaluated in all blood and MacConkey agar testing. **Table 3** lists the non-target resistance mechanisms that were evaluated for cross reactivity. All 81 non-target organisms (100%) resulted in a negative NG-Test CARBA 5 result from blood and MacConkey agar. 54/81 (66.7%) of these non-target organisms were *Enterobacteriaceae*, 20/81 (24.7%) were *P. aeruginosa*, and 7/81 (8.6%) were *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria monocytogenes*, *Stenotrophomonas maltophilia*, and *Corynebacterium jeikeium*. 15 non-target *Enterobacteriaceae* were tested from HC CRE agar and all (100%) were negative on NG-Test CARBA 5 as expected.



Conclusions

Knowing the type of carbapenemase is crucial for therapeutic purposes, infection control, and for preventing outbreaks because some carbapenemases are plasmid-mediated and can thus spread through hospitals or networks of hospitals, leading to epidemic spread of AMR genes. Additionally, more recent FDA approved drugs have been shown to only be effective against particular carbapenemases but not others. For example, Meropenem-Vaborbactam has been shown to treat KPC-producing *Enterobacteriaceae*, but not OXA-48 or metallo-beta-lactamase producing bacteria. Prescribing the right antibiotic is important for the patient and to reduce costs. In these instances, using a rapid lateral flow assay is incredibly useful as a quick, easy to use and low cost test to confirm the carbapenemase phenotype in the clinical lab setting. It requires no additional equipment which facilitates rapid implementation. Due to the global spread of carbapenemases, rapid genotypic assays are often employed or encouraged for CPO detection; however molecular tests can be costly and must be paired with phenotypic test results. NG-Test CARBA 5 offers a quick and cost-effective method to identify the 5 most common carbapenemases from cultures when a CPO is suspected.

FDA clearance pending