Evaluation of the NG-Test CARBA 5 for the Phenotypic Detection of Carbapenemases

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INTRODUCTION

As carbapenem-resistant *Enterobacterales* continue to proliferate in the U.S. and throughout the world, rapid and reliable detection of the carbapenemases they produce is a pressing need. Hardy Diagnostics NG-Test CARBA 5 is a rapid, simple, and visual multiplex lateral flow immunochromatographic assay for the detection of the five common carbapenemases (KPC, OXA-48-like, VIM, IMP, and NDM). The test utilizes labeled monoclonal antibodies against the 5 carbapenemases to detect the presence of these enzymes in an organism after incubation in a lysis buffer. Our objective was to evaluate the performance of the Hardy NG-Test CARBA 5 for sensitivity and specificity when tested using isolates cultured on various media types and includes *Enterobacteriaceae* and *Pseudomonas* species.

MATERIALS AND METHODS

Isolates.
50 Isolates From the Washington University strain bank
- Barnes Jewish Hospital (n=34)
- Urine and hospital environmental strains from specimens from Pakistan (n=16)

60 Isolates from Hardy Diagnostics

Test Conditions.
16-24 hours subculture with an ertapenem disk between the 3rd and 4th quadrant
- TSA with 5% blood (BAP)
- MacConkey Agar (MAC)

CLSI guidelines for antimicrobial susceptibility testing with ertapenem
- Mueller Hinton Agar (MH)

Carbapenemase inactivation method was performed on all isolates

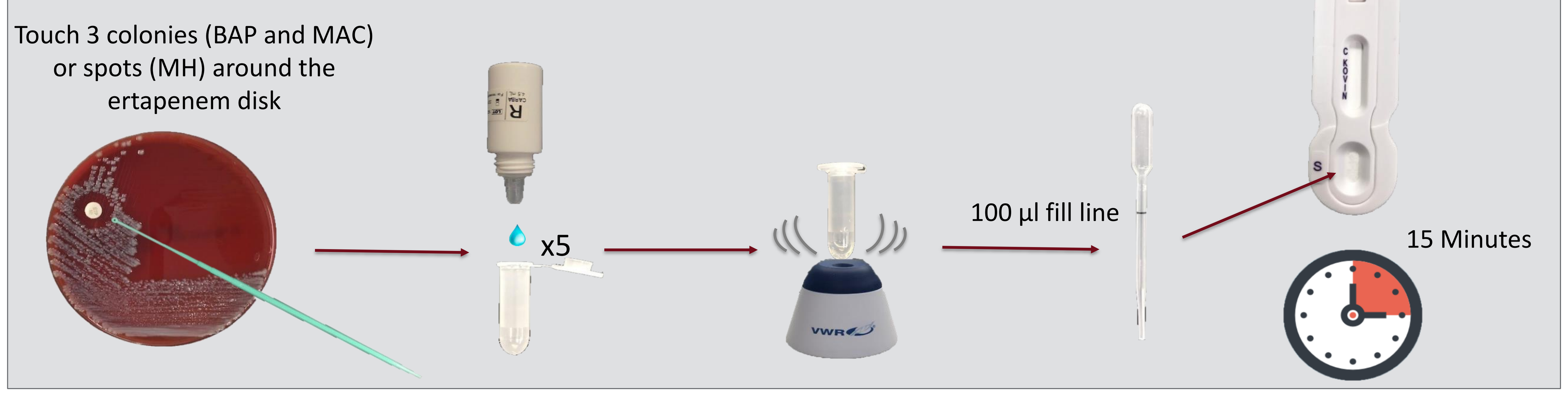
Quality Control. Quality control (QC) with the listed organisms was performed on every day of testing. The same processing used for test isolates was followed for QC organisms.

QC Strain	Expected Result
Klebsiella pneumoniae ATCC BAA-1705	Positive for KPC
Klebsiella pneumoniae NCTC 13442	Positive for OXA-48-like
Klebsiella pneumoniae NCTC 13439	Positive for VIM
Escherichia coli NCTC 13476	Positive for IMP
Escherichia coli ATCC BAA-2146	Positive for NDM
Klebsiella pneumoniae ATCC BAA-1706	Negative

Comparator Methods. Each isolate had a genotypic resistance mechanism previously determined using molecular methods (PCR, Gene Xpert Carba-R, or whole genome sequencing (WGS)).

MATERIALS AND METHODS

Figure 1. NG-Test CARBA 5 Processing



RESULTS

Table 1. Resistance Mechanism Detection by NG-Test CARBA 5 on BAP

Resistance Mechanism by Reference Method	NG-Test CARBA 5 Result: KPC	OXA	VIM	IMP	NDM	NDM+OXA	OXA+VIM	Negative	Grand Total
KPC	26								26
OXA		15							15
VIM			8						8
IMP				9				2	11
NDM					27				27
NDM+OXA						3			3
OXA+VIM							1		1
Negative								19	19
Grand Total	**26**	**15**	**8**	**9**	**27**	**3**	**1**	**21**	**110**

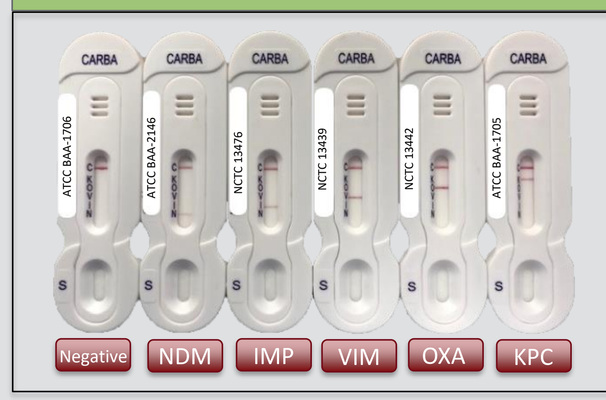
Table 2. Resistance Mechanism Detection by NG-Test CARBA 5 on MAC

Resistance Mechanism by Reference Method	NG-Test CARBA 5 Result: KPC	OXA	VIM	IMP	NDM	NDM+OXA	OXA+VIM	Negative	Grand Total
KPC	26								26
OXA		15							15
VIM			8						8
IMP				10				1	11
NDM					27				27
NDM+OXA						3			3
OXA+VIM							1		1
Negative								19	19
Grand Total	**26**	**15**	**8**	**10**	**27**	**3**	**1**	**20**	**110**

Table 3. Resistance Mechanism Detection by NG-Test CARBA 5 on MH

Resistance Mechanism by Reference Method	NG-Test CARBA 5 Result: KPC	OXA	VIM	IMP	NDM	NDM+OXA	OXA+VIM	Negative	Grand Total
KPC	26								26
OXA		15							15
VIM			8						8
IMP				8				3	11
NDM					27				27
NDM+OXA						3			3
OXA+VIM							1		1
Negative								19	19
Grand Total	**26**	**15**	**8**	**8**	**27**	**3**	**1**	**22**	**110**

Figure 2. Quality Control Test Organisms



Test Line Key
C= Control
K= KPC
O= OXA-48-like
V= VIM
I= IMP
N= NDM

Figure 3. Isolates with >1 Mechanism Detected

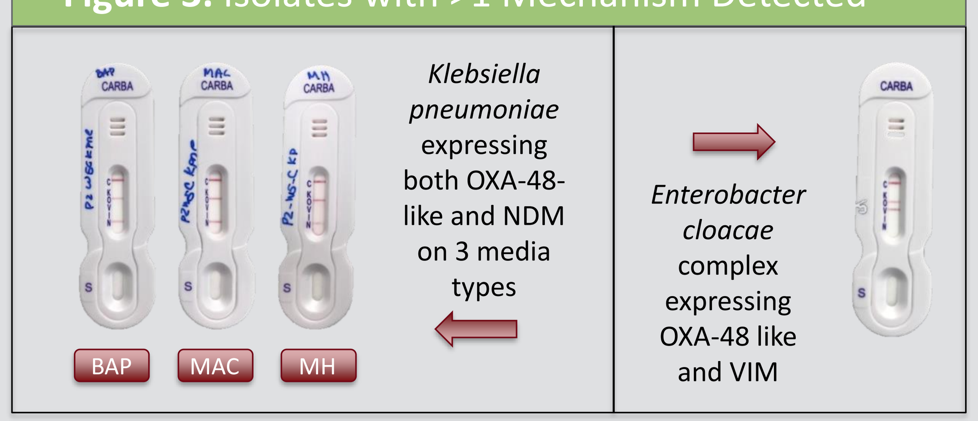
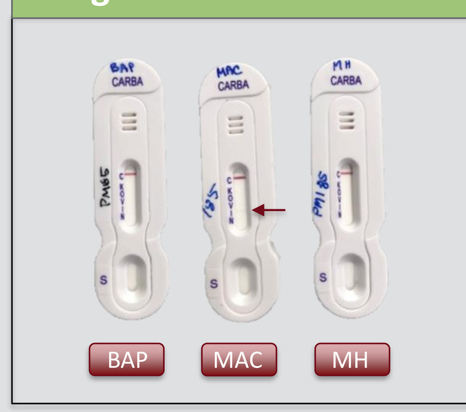


Figure 4. IMP-27



An example of a *Proteus mirabilis* isolate with IMP-27 that was weakly positive for IMP on MAC only.

RESULTS

Table 4. Sensitivity and Specificity by Organism Type

BAP	n	True Positive	False Positive	True Negative	False Negative	Sensitivity	Specificity
Klebsiella	37	34	0	3	0	100.00%	100.00%
Enterobacter	23	16	0	7	0	100.00%	100.00%
Pseudomonas	9	4	0	5	0	100.00%	100.00%
Other *Enterobacteriaceae*	41	35	0	4	2	94.59%	100.00%
Total	**110**	**89**	**0**	**19**	***2**	**97.80%**	**100.00%**
MAC	**n**	**True Positive**	**False Positive**	**True Negative**	**False Negative**	**Sensitivity**	**Specificity**
Klebsiella	37	34	0	3	0	100.00%	100.00%
Enterobacter	23	16	0	7	0	100.00%	100.00%
Pseudomonas	9	4	0	5	0	100.00%	100.00%
Other *Enterobacteriaceae*	41	36	0	4	1	97.30%	100.00%
Total	**110**	**90**	**0**	**19**	**+1**	**98.90%**	**100.00%**
MH	**n**	**True Positive**	**False Positive**	**True Negative**	**False Negative**	**Sensitivity**	**Specificity**
Klebsiella	37	34	0	3	0	100.00%	100.00%
Enterobacter	23	16	0	7	0	100.00%	100.00%
Pseudomonas	9	4	0	5	0	100.00%	100.00%
Other *Enterobacteriaceae*	41	34	0	4	3	91.89%	100.00%
Total	**110**	**88**	**0**	**19**	**#3**	**96.70%**	**100.00%**

* *Proteus mirabilis* (n=2) with IMP-27
+ *Proteus mirabilis* (n=1) with IMP-27
# *Proteus mirabilis* (n=2) and *Providencia rettgerii* (n=1) with IMP-27

CONCLUSIONS

- The NG-Test CARBA 5 identified carbapenemases with 97.8%, 98.9%, and 96.7% sensitivity on BAP, MAC, and MH agar and 100% specificity on all media types.

- All resistance genes were detected with the exception of 3 strains with IMP-27 (identified by WGS) that were variably detected with the NG-Test CARBA 5 assay. IMP-27 detection varied across media; 1/3 was detected on BAP, 2/3 on MAC, and 0/3 were detected on MH agar.

- Notably, strains with >1 carbapenemase (OXA + VIM (n=1), OXA + NDM (n=3)) demonstrated 100% correlation between the NG-Test CARBA 5 and reference methods.

- The NG-Test CARBA 5 is sensitive and specific for KPC, OXA-48-like, VIM, IMP, and NDM. With a 15 minute incubation period, it can easily be performed in real time on the bench when resistance to a carbapenem is first detected and could streamline workflow.

Acknowledgements

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