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Implementation of a Rapid, Multiplex Immunochromatographic Assay to Streamline Culture-based Colonization Screenings for Carbapenemase-Producing Organisms

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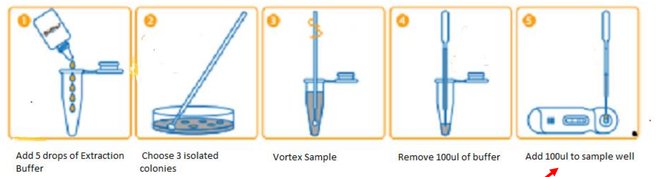
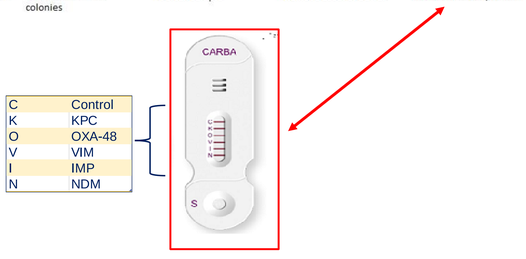
Overview

- Carbapenem antibiotics are some of the last line of defense against hard to treat, resistant gram-negative bacteria. β-lactamases, specifically the Big 5 carbapenemase enzymes (KPC, NDM, OXA-48, VIM, and IMP), hydrolyse these antibiotics rendering them ineffective for treatment. Detection of these carbapenemases or their genes (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48-like}$, *bla*$_{VIM}$ and *bla*$_{IMP}$), is necessary for patient care as well as infection control.
- Most carbapenemase-producing organisms (CPO) are considered urgent antimicrobial resistance (AR) threats according to the Centers for Disease Control and Prevention (CDC's) *Antibiotic Resistance Threats in the United States 2019 Report* and a serious concern for patients in healthcare facilities.
- Current PCR methods that detect the Big 5 carbapenemase genes can be labor and time intensive, require equipment purchases and maintenance, and are costly.
- The NG-Test® CARBA 5 (CARBA 5) is an in vitro, rapid, multiplex immunochromatographic assay that detects the Big 5 carbapenemase enzymes and is FDA-approved for Enterobacterales and *Pseudomonas aeruginosa* isolates. (Figure 1.)
- As the Northeast Regional AR Laboratory, Wadsworth Center (WC) identifies and characterizes CPO isolates recovered from colonization screenings (CS). These screenings are triggered by clinical cases in healthcare facilities and typically include rectal swab collections of close contacts to assess issues in infection control.
- Understanding the carbapenemase enzyme and carbapenemase gene variants detected by these methods can be important to understanding detection of clinical and colonization cases.
- From January 2021 to April 2023 WC has tested >9000 rectal swabs with 15% (1300) determined to be positive for a carbapenemase gene and requiring culture isolation to definitively determine if transmission has occurred.
- A CARBA 5 verification study of 120 isolates demonstrated 96% accuracy, 95% sensitivity, and 100% specificity.
- Incorporation of the CARBA 5 test into our culture-based colonization screening algorithm requires less hands-on processing time without additional equipment costs or maintenance, decreases turnaround time (TAT) to result and is less expensive. (Figure 2.)

Procedure – NG-TEST® CARBA 5

- A liquid extraction buffer lyses the bacterial cells.
- 100µl of sample is then added to the test cartridge.
- Capillary action of the test cartridge pulls the sample through the nitrocellulose membrane with the immobilized monoclonal antibodies for 5 carbapenemase enzymes (KPC, NDM, OXA-48, VIM, and IMP).
- The immobilized control antibodies capture any mobile antibodies that run through the test strip that do not bind to the five targets.





CARBA 5 Result Interpretation

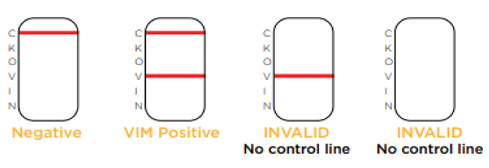


Figure 1.

https://hardydiagnostics.com/

CPO Screening Algorithm

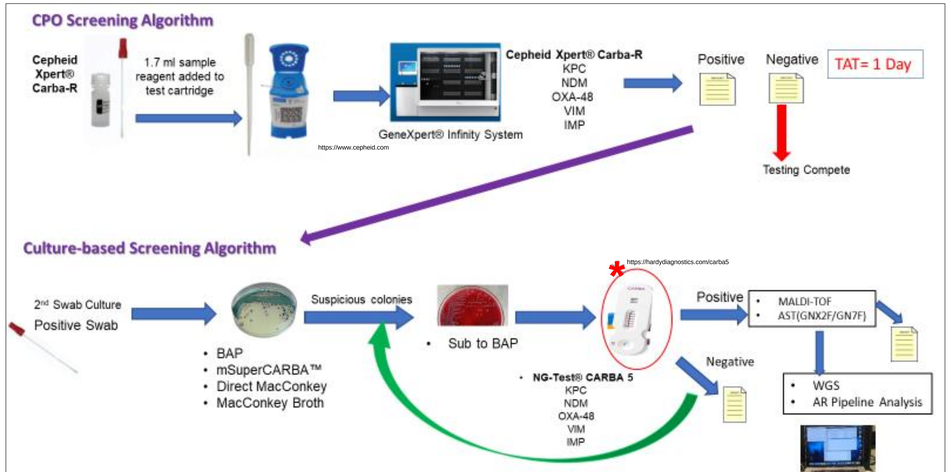
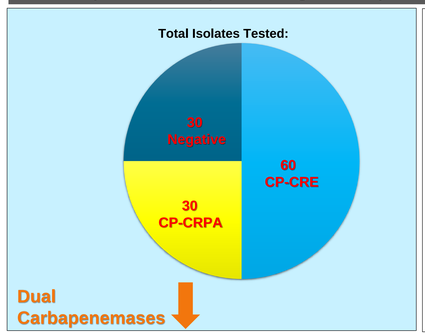
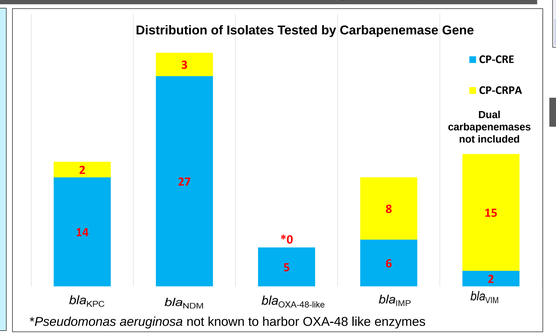


Figure 2.

- Incorporating CARBA 5 testing in place of real-time PCR for the Big 5 can decrease TAT by 1 day.
- CARBA 5 replaces the need for expensive real-time PCR instrumentation, service and maintenance.
- CARBA 5 requires less high complexity testing training compared to the training and expertise needed for real-time PCR.

Results – Carbapenemase-producing Carbapenem-resistant Enterobacterales (CP-CRE) Carbapenemase-producing Carbapenem-resistant *Pseudomonas aeruginosa* (CP-CRPA)

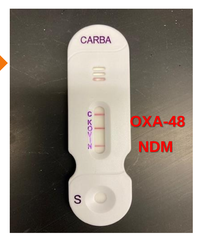




Pseudomonas aeruginosa not known to harbor OXA-48 like enzymes

Enterobacterales	Dual Carbapenemases
Klebsiella pneumoniae (2)	OXA-232*/NDM-1
Klebsiella pneumoniae	OXA-232*/NDM-5
Enterobacter cloacae complex (2)	KPC-4/NDM-7
Enterobacter cloacae complex	KPC-3/NDM-1
Pseudomonas aeruginosa	Dual Carbapenemases
Pseudomonas aeruginosa	NDM-1/VIM-5
Pseudomonas aeruginosa	NDM-1/IMP-26

CARBA 5 Dual Carbapenemase Detection



- 6/60 Enterobacterales contain dual carbapenemases
- 2/30 *Pseudomonas aeruginosa* dual contain carbapenemases
  *OXA-232 is an OXA-48 like carbapenemase

Carbapenemase Gene Variants Tested

NG-Test® CARBA 5 Carbapenemase Variants Tested		Variants DETECTED	Variants known NOT TO BE DETECTED *
Enterobacterales	KPC	2, 3, 4, 5, ,6 ,9, 12 ,23	
	OXA-48 like	48, 162, 163, 181, 204, 232, 244, 245, 370, 405, 436, 484, 517, 519, 535	
	VIM	1, 2, 4, 5 , 6, 19, 23, 26, 27, 31, 39, 46, 51, 52, 54, 56, 58, 59	
	IMP	1, 4, 6, 7, 8, 10, 11, 22, 26, 29	14, 27
	NDM	1, 2, 3, 4, 5, 6, 7, 8, 9	
Pseudomonas aeruginosa	KPC	5	
	OXA-48 like	181	
	VIM	2 ,4, 5, 11, 80	
	IMP	1, 4, 7, 8, 14, 19, 26	13, 14, 18
	NDM	1	

- The CARBA 5 immunochromatographic assay was successful in detecting all available carbapenemase gene variants except IMP variants 13, 14, 18, and 27. Non-detection of IMP 14 is a known limitation of the assay.
- Variants in RED were provided by WC.
  * Not all carbapenemase gene variants were available for testing.

Method Comparison

Test	Method	Time to Result	Instrumentation Required?	Molecular Expertise Required	Overall Cost
NG-Test® CARBA 5*	Immunochromatographic	15 minutes	No	No	↓ $24
Xpert® Carba-R*	Automated Real-time PCR	1 hour	Yes	Somewhat	↑ $45
LDT* Real-time PCR	Real-time PCR	4 to 6 hours	Yes	Yes	↑ $10

* Both the CARBA 5 and Carba-R tests have known limitations in detection of IMP variants. This should be considered when performing screenings for IMP carbapenemases.

Discussion

- The CARBA 5 test offers a low-cost, rapid alternative to identify the non-IMP Big 5 carbapenemase enzymes in our culture-based colonization screening algorithm.
- The CARBA 5 test does not require instrumentation, maintenance or molecular expertise to perform.
- There are known limitations to the detection of IMP variants (13, 14, 18, 27) with this method.
- No false positive results were obtained when testing Enterobacterales and *Pseudomonas aeruginosa*.
- The CARBA 5 was successful in detecting dual carbapenemases in 8 CPOs tested.
- To further complement our culture-based screening algorithm, we are evaluating the utility of CARBA 5 for the detection of the Big 5 carbapenemase enzymes from *Acinetobacter baumannii* isolates. Preliminary data demonstrate false positive detection of IMP in some isolates.
- The CARBA 5 test can be instituted in low complexity settings with limited funding for the detection of the Big 5 carbapenemase enzymes to support the detection of colonization cases in healthcare facilities to assess transmission of AR threats and support infection control investigations.

Acknowledgements

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