Evaluation of Testing Methods for Carbapenem Resistant Gram-Negative Bacilli

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INTRODUCTION

Carbapenem Resistant *Enterobacteriaceae* (CRE) are becoming an increasing global health threat and a great concern to healthcare providers worldwide. Efficient and robust screening methods for detecting CRE are in demand to contain the spread of infections between patients. CRE can be detected through automated antimicrobial susceptibility testing (AST), phenotypic, and molecular methods. However, there are limitations to these methods. Our study will aid in rapid and accurate identification of CRE carriers, as well as increase sensitivity and specificity of current confirmatory test methods. The study involved the (1) validation of 2010 CLSI carbapenem and cephalosporin antimicrobial breakpoints on the Vitek®2 which gave 100% categorical agreement for cefazolin while the remaining drug classes had multiple major and very major errors, (2) verifying the use of the mCIM for screening for CRE, which of 19 known CDC isolates tested, specificity of 100% and sensitivity of 63.64% were reported, and (3) validation of rectal swab specimens for the detection of CRE on the Cepheid® GeneXpert CARBA-R assay which resulted in 100% sensitivity and specificity for the detection of five carbapenemase genes. In conclusion, 2010 CLSI carbapenem and cephalosporin antimicrobial breakpoints for the Vitek®2 will require further testing; mCIM may be used as a screening method, and rectal swabs are an appropriate specimen type for carbapenemase gene detection on the Cepheid® GeneXpert.

METHODS

Vitek® 2 (bioMérieux) AST for Cephalaproins and Carbapenems.

Bacterial Isolates: Thirty-Five *Enterobacteriaceae* frozen isolates were recovered at AUMC

Procedure: Vitek® 2 AST testing was performed using AST-GN69 and AST-XN06 cards (bioMérieux, Durham, NC), according to the manufacturer's instructions.

Modified Carbapenem Inactivation Method (mCIM).

Bacterial Isolates: Twenty-seven isolates (16 *Enterobacteriaceae*, 4 *Pseudomonas aeruginosa,* and 7 *Acinetobacter baumannii).*

Procedure: The mCIM procedure modified by McMullen et al. (2017) was used. The negative control was a meropenem disk incubated in TSB with no organism. Zones were measured after overnight incubation (18-24 hours) at 35 °C.

Analysis of Rectal Swabs as Specimen Type on Cepheid GeneXpert CARBA R.

Bacterial Isolates: Five negative stool samples were prepared. Ten mock swab samples positive for one of five known organisms carrying the following carbapenemase genes detected by the assay: bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{KPC}, *and* bla_{OXA-48}.

Procedure: All specimens were run using the Xpert Carba-R cartridge and analyzed using the Cepheid GeneXpert according to the manufacturer's instructions.

RESULTS

Table 1. Calculated categorical agreement, very major, and major errors from validation of carbapenem and cephalosporin antimicrobial breakpoints on Vitek®2.

	Cefazolin	Cefotaxime	Ceftriaxone	Ceftazidime	Ertapenem	Imipenem	Meropenem
Categorical Agreement	100%	85.29%	94.12%	85.29%	73.53%	82.35%	91.18%
Major Errors	0%	5.88%	0%	2.94%	5.88%	11.76%	2.94%
Very Major Errors	0%	8.82%	5.88%	11.76%	20.59%	5.88%	5.88%

Table 2. modified Carbapenem Inactivation Method (mCIM) Results.

Vitek® MALDI TOF MS Identification	Cepheid® Gene Xpert CARBA-R Gene Detected	mCIM Zone Size (mm)	mCIM Zone Interpretation
E. coli	KPC	6	Resistant
E. coli	NDM	6	Resistant
K. pneumoniae	OXA	24	Susceptible
K. pneumoniae	NDM	20	Susceptible
K. pneumoniae	ND*	6	Resistant
K. pneumoniae	KPC	6	Resistant
S. marcescens	SME	6	Resistant
R. ornithinolytica	KPC	6	Resistant
C. wermanii	NDM	6	Resistant
E. cloacae	IMP	25	Susceptible
K. pneumoniae	NEG	28	Susceptible
K. pneumoniae	NEG	27	Susceptible
K. pneumoniae	NEG	33	Susceptible
E. cloacae	NEG	24	Susceptible
E. cloacae	NEG	23	Susceptible
E. cloacae	NEG	27	Susceptible
A. baumannii	ND*	6	Resistant
A. baumannii	ND*	6	Resistant
A. baumannii	ND*	27	Susceptible
A. baumannii	ND*	6	Resistant
A. baumannii	OXA-23	6	Resistant
A. baumannii	NEG	19	Susceptible
A. baumannii	ND*	27	Susceptible
P. aeruginosa	ND*	6	Resistant
P. aeruginosa	NEG	27	Susceptible
P. aeruginosa	VIM	28	Susceptible
P. aeruginosa	ND*	6	Resistant

ND*: Not determined

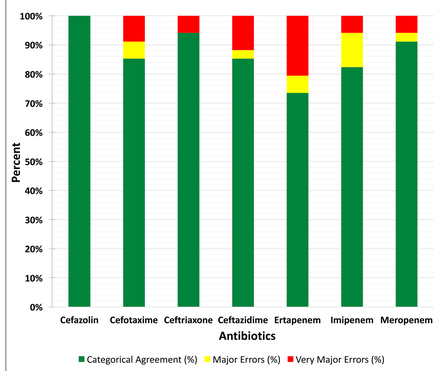

Figure 1. Bar graph representing calculated categorical agreement, very major, and major error data from validation of carbapenem and cephalosporin antimicrobial breakpoints on Vitek 2®.

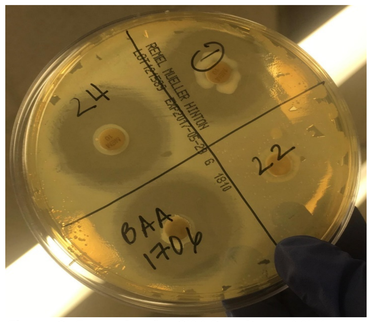

Figure 2. Example of mCIM Results on MHA. Sample 24 is positive, (-) is negative, BAA 1706 is negative, and 22 is positive. A positive result was a zone diameter of <8 mm, and a negative result was a zone diameter >15 mm. A zone diameter of 9-14 mm is indeterminate.

Table 3. Detection of CRE on Cepheid ® GeneXpert Using Rectal Swabs.

Specimen ID	IMP	VIM	NDM	KPC	OXA48
CARBA-R POS Control	+	+	+	+	+
CARBA-R NEG Control	-	-	-	-	-
NEG Patient 2353	-	-	-	-	-
NEG Patient 2285	-	-	-	-	-
NEG Patient 3589	-	-	-	-	-
NEG Patient 1402	-	-	-	-	-
NEG Patient 2112	-	-	-	-	-
Mock Stool A					
MSA CDC103- PAER IMP	+	-	-	-	-
MSA CDC54- PAER VIM	-	+	-	-	-
MSA CDC137- ECOL NDM	-	-	+	-	-
MSA CDC136- ECLO KPC	-	-	-	+	-
MSA CDC160- KPNE OXA48	-	-	-	-	+
Mock Stool B					
MSB CDC103- PAER IMP	+	-	-	-	-
MSB CDC54- PAER VIM	-	+	-	-	-
MSB CDC137- ECOL NDM	-	-	+	-	-
MSB CDC136- ECLO KPC	-	-	-	+	-
MSB CDC160- KPNE OXA48	-	-	-	-	+

CONCLUSIONS

Validation of CLSI M100-S24 breakpoints to identify carbapenem and cephalosporin antimicrobial susceptibilities on Vitek®2 AST.

1. Most of the discrepancies are seen in the carbapenem class of antimicrobials.
2. Further testing, with more isolates, is needed to validate updated CRE breakpoints on the Vitek®2.

Validation of the mCIM for phenotypic screening of CRE .

1. The mCIM is accurate in detecting other clinically significant gram-negative bacilli.
2. The mCIM may be used with Vitek® 2 as a way to improve CRE identification

Detection of CRE on Cepheid ® GeneXpert Using Rectal Swabs.

1. Rectal swabs can be used as a reliable specimen type on the Cepheid GeneXpert ® CARBA R assay.
2. Rectal swabs can be implemented as a screening tool.

CLINICAL IMPLICATIONS

Our evaluation will:

- Improve lab efficiency by implementing a workflow that will allow for the rapid and accurate identification of CRE carriers in the clinical setting.
- Increase the sensitivity and specificity of current confirmatory test methods.
- Assist in infection control.
- Aid in antimicrobial stewardship.

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