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Comparison of Sensititre®Aris 2X and bioMérieux Vitek® 2 Systems for Detection of *Klebsiella pneumoniae* and *Enterobacter cloacae* Carrying the KPC Beta-lactamase

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ABSTRACT

Enterobacteriaceae that harbor bla_{KPC} have rapidly become a significant clinical problem in the United States and worldwide. These bacteria are resistant to all beta-lactam antimicrobials, including carbapenems, severely limiting treatment options. The KPC (*Klebsiella pneumoniae* carbapenemase) gene is plasmid-mediated, facilitating rapid spread within and between patients. When assessing clinical cultures, accurate detection of bacteria that may carry bla_{KPC} by routine methods used in the clinical microbiology lab is vital to appropriate antibiotic therapy and infection control intervention. Incorporating an optimized inoculum for the Sensititre system, we compared the automated Sensititre Aris 2X (S) and Vitek 2 (V) antimicrobial susceptibility systems to PCR for detection of *Enterobacteriaceae* that contain bla_{KPC}. Archived isolates taken from a collection at Albany Medical Center included 90 *K. pneumoniae* (70 KPC positive; 20 KPC negative) and 60 *E. cloacae* (28 KPC positive; 32 KPC negative). All strains had reduced susceptibility to cephalosporin and/or carbapenem antibiotics by disk diffusion. By CLSI guidelines isolates with an MIC of greater than or equal to 2 µg/ml to ertapenem, imipenem or meropenem may contain a KPC. Using these guidelines, the sensitivity of detection of a possible KPC was 98% for Sensititre and 98% for Vitek when testing was performed with ertapenem. Sensitivities for S and V, respectively were 97% and 84% with imipenem and 91% and 69% with meropenem. Of 52 KPC negative isolates 32 (61% specificity) had an MIC ≤ 1 µg/ml to ertapenem by S and 21 (40%) by V. Specificities for S and V, respectively were 85% and 94% with imipenem and 87% and 96% with meropenem. Both the optimized S and V methods had very good sensitivity with ertapenem, but S was more sensitive for detection of a KPC with either imipenem or meropenem. S was more specific when testing ertapenem, but V more specific with imipenem or meropenem. Both methods may be relied upon for detection of isolates with bla_{KPC}.

INTRODUCTION

Detection of carbapenemase-production in *Enterobacteriaceae* is essential for selection of appropriate antibiotic therapy and initiation of infection control measures. Clinical laboratories rely on results of routine susceptibility test methods to indicate species that may harbor KPCs. Although isolates may test within the susceptible range according to CLSI breakpoints, some may contain KPCs. Resistance to one or more extended spectrum cephalosporins with reduced susceptibility to ertapenem have been shown to be the most sensitive indicators of a KPC. Isolates with an ertapenem MIC ≥ 2 µg/ml (or an imipenem or meropenem MIC of 2 – 4 µg/ml) should be further screened for the presence of a KPC using either PCR or the Modified Hodge Test.

In this evaluation we tested a collection of KPC-producing *K. pneumoniae* (Kpn) and *E. cloacae* (Eclo) with two automated susceptibility test systems used in clinical microbiology laboratories, the TREK Aris 2X Sensititre and bioMérieux Vitek 2. An optimized inoculum was introduced to enhance detection with the Sensititre system. A major goal was to ascertain if each of these systems would identify isolates that may contain KPCs by evaluating MICs for carbapenems for isolates known to be positive for bla_{KPC} by PCR. Isolates that had reduced susceptibility to cephalosporins and/or carbapenems but were negative for bla_{KPC} were included to assess test specificity.

In 2010 the CLSI has proposed lowering carbapenem breakpoints as shown in Table 1. More isolates will be reported as resistant and the need for supplemental confirmatory testing for KPCs will be reduced. However, these changes will increase reliance on accurate MIC results. An additional goal of this study was to compare the range of MICs for KPC-producing and non-KPC strains using the two test methods.

MATERIALS AND METHODS

Bacterial Isolates:
Kpn and Eclo were from an archived collection derived from clinical and surveillance cultures performed at Albany Medical Center (AMC). Isolates were stored at -70°C in Trypticase Soy broth with 10% glycerol. All strains had reduced susceptibility to cephalosporin and/or carbapenem antibiotics (meropenem ≤ 25 mm or ertapenem ≤ 18 mm) by disk diffusion. Twelve Kpn were carbapenem susceptible ESBLs.

Isolates included 90 Kpn (70 KPC positive; 20 KPC negative) and 60 Eclo (28 KPC positive; 32 KPC negative), as determined by PCR. Isolates were subcultured to Trypticase soy blood agar (TSB) and passed to TSB again prior to susceptibility testing.

Real-time PCR:
Testing for bla_{KPC} was performed at AMC on a SmartCycler instrument using a method adapted from Vincent Lombombardi, Mt. Sinai Hospital, NYC. One colony was suspended in 1 ml of nuclease-free water. Master mix contained 0.8 µM each primer, 1X QuantiTect multiplex mix and 3 µl colony suspension per 25 µl reaction. Primers: KPC721F GGC ACG GCA AAT GAC TAT G KPC 888R GCC AAT AGA TGA TTT TCA GAG C Probes: KPC3 TET-AAG GAT GAC AAG TAC AGC GAT CCT T-B HQ1 KPC 1,2,4 FAM-AAG GAT GAC AAG CAC AGC GAT CCT T-B HQ1. Cycling conditions: 10 min at 95°C, followed by 40 Cycles: 15 sec at 95°C and 30 sec at 60°C.

Vitek 2 Susceptibility:
Bacteria were suspended in 0.45% saline to a 0.5 McFarland standard. A TSB plate was streaked for purity. 145 µl of standardized suspension was diluted into 3 ml saline for testing with the GN20 card in a Vitek 2 Compact Instrument according to manufacturer's instructions.

Sensititre Susceptibility:
Isolates were suspended in sterile water to a 0.5 McFarland standard. A 30µl inoculum of standardized suspension was transferred to 11 ml of Mueller Hinton broth. Using the Sensititre Autoinoculator 50 µl of suspension (~4 x 10^5 CFU/ml) was inoculated into a GNXF susceptibility panel. The positive control well was sampled to assess purity and verify the inoculum concentration. Panels were incubated for 18 hours in the Aris 2X incubator before an autoread was performed according to the software protocol.

Statistical Analysis
Geometric means and the Mann Whitney U test statistics were calculated with SPSS 13 software.

Table 1. CLSI Breakpoints for Carbapenems

	2009 CLSI (M100-S19)			2010 Proposed CLSI (M100-S20 Supplement)		
Carbapenem	Susc	Interm	Resist	Susc	Interm	Resist
Ertapenem	≤ 2	4	≥ 8	≤ 0.25	0.5	≥ 1
Meropenem	≤ 4	8	≥ 16	≤ 1	2	≥ 4
Imipenem	≤ 4	8	≥ 16	≤ 1	2	≥ 4
Doripenem	≤ 0.5*		≥ 1*	≤ 1	2	≥ 4

*FDA breakpoints. No CLSI breakpoints assigned.

Table 2. Number of Isolates at each MIC for Carbapenems Tested with Sensititre Aris 2X (S) and Vitek 2 (V)

KPC PCR Pos (n=98)		MIC µg/ml												
	Reportable Range	0.125	0.25	0.5	1	2	4	8	16	>16	MIC ≥2 (%)	MIC_{50}	MIC_{90}	Geom Mean
Ertapenem - S	1-16				2	10	17	36	23	10	96 (98)	8	>16	8.0*
Ertapenem - V	0.5-8			2			16	80			96 (98)	8	8	6.7
Meropenem - S	1-8				9	11	19	38	21		89 (91)	8	>8	5.7φ
Meropenem - V	.25-16			4	26	42	5	8	13		68 (69)	2	16	2.3
Imipenem - S	1-8				3	6	19	53	17		95 (97)	8	>8	6.8φ
Imipenem - V	1-16				16	26	23	18	15		82 (84)	4	16	3.7
Doripenem - S	0.125-2		1	2	15	27	53				80 (82)	4	4	ND
KPC PCR Neg (n=52)		MIC µg/ml												
	Reportable Range	0.125	0.25	0.5	1	2	4	8	16	>16	MIC ≤ 1 (%)	MIC_{50}	MIC_{90}	Geom Mean
Ertapenem - S	1-16				32	6	6	6	1	1	32 (62)	≤ 1	8	1.8
Ertapenem - V	0.5-8			20	1	13	11	7			21 (40)	2	8	1.6
Meropenem - S	1-8				45	4	2		1		45 (87)	≤ 1	2	1.2φ
Meropenem - V	.25-16		44	2	4	1				1	50 (96)	≤ 0.25	1	0.3
Imipenem - S	1-8				44	5	2	1			44 (85)	≤ 1	2	1.7
Imipenem - V	1-16				49	2		1			49 (94)	≤ 1	≤1	1.1
Doripenem - S	0.125-2	29	5	4	10	2	2				48 (92)	≤ 0.12	1	ND

Notes for Table 2:
1. Proposed CLSI 2010 Breakpoints Susceptible ☐ Intermediate ▒ Resistant ■
2. Test panels for ertapenem did not include the proposed CLSI 2010 susceptible and intermediate range for S, nor the susceptible range for V. Since the conclusion of this study, TREK has introduced standard MIC products including ertapenem ranges starting at 0.25 µg/ml.
3. Organism that grew at the highest dilution on the panel are reported at the next doubling dilution. When no growth was observed at the lowest dilution tested, the MIC is recorded as the lowest dilution tested.
4. When the MIC_{50} results for KPC negative strains are the same as the lowest dilution tested they are reported as less than or equal to that dilution.
5. Doripenem was not available on Vitek 2.
6. *P value for difference of geometric means = 0.04. φP value for difference of geometric means <0.001.

RESULTS

Ertapenem (Table 2):

1. As suggested by 2009 CLSI guidelines, when an ertapenem MIC ≥ 2 µg/ml was used as the indicator of a possible KPC, sensitivity of detection was equivalent for S (98%) and V (98%).
2. At the time of this analysis panels did not include dilutions <1 µg/ml for S and <0.5 µg/ml for V. The number of KPC negative isolates that would be reported as resistant to ertapenem by 2010 CLSI guidelines could not be determined.
3. Using an MIC ≤ 1 µg/ml as a break-point for lack of presence of a KPC, S was more specific (62%) than V (40%).

Meropenem, Imipenem, Doripenem (Table 2):
Using proposed 2010 CLSI guidelines (MIC ≥ 2 µg/ml is non-susceptible)

1. Classification as Non-Susceptible for KPC-producing isolates:
   Sensititre: meropenem 91%
   imipenem 97%
   Vitek 2: meropenem 69%
   imipenem 84%
2. Classification as Susceptible for KPC-non-producing isolates:
   Sensititre meropenem 87%
   imipenem 85%
   VItek 2: meropenem 96%
   imipenem 94%
3. KPC positive isolates: The MIC_{50} was 1 or 2 dilutions higher for imipenem and meropenem, respectively when tested with S compared to V. The MIC_{90} was essentially the same for both methods.
4. KPC negative isolates: The MIC_{50} could not be accurately determined due to lack of testing at low concentrations, but is generally ≤ 1 µg/ml. The MIC_{90} was higher for ertapenem than for other carbapenems. The MIC_{90} was 1 dilution lower for meropenem and imipenem when tested with V compared to S.
5. Results for doripenem with S: 82% of KPC positive isolates were non-susceptible; 92% of KPC negative isolates were susceptible.

CONCLUSIONS

1. Sensititre ARIS 2X and Vitek 2 have equivalent sensitivity for detection of a possible KPC if an ertapenem MIC of ≥ 2 µg/ml is used as an indicator. Ertapenem was the most sensitive indicator of a KPC.
2. For meropenem and imipenem both systems did not report all KPC-producing strains within non-susceptible categories. This underscores the need for ertapenem susceptibilities and KPC confirmatory testing for implementation of infection control measures.
3. When testing with meropenem and imipenem, MIC results by the ARIS 2X System are more sensitive and Vitek 2 are more specific for correlation with the presence of a KPC. In this study Vitek 2 significantly under-called resistance for these carbapenems compared to the ARIS2X.
4. As lower breakpoints for carbapenems are adopted, it is important that susceptibility test methods accurately determine resistance, especially if KPC confirmatory testing is not performed and dosing is dependent on MIC.

REFERENCES

1. Anderson K, D Lonsway, J Rasheed, J Biddle, B Jensen, L McDougal, R Carey, A Thompson, S Stocker, B Limbago, J Patel Evaluation of methods to identify the Klebsiella pneumoniae carbapenemase in Enterobacteriaceae. J Clin Microbiol. 2007 Aug;45(8):2723-5.
2. Clinical and Laboratory Standards Institute. 2009. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
3. Clinical and Laboratory Standards Institute. 2010 Teleconference Series. CLSI 2010 AST Update. J. Hindler. Feb 2010.