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Performance of Vitek 2 for Antimicrobial Susceptibility Testing of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* with Vitek 2 (2009 FDA) and CLSI M100S 26th Edition Breakpoints

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ABSTRACT The performances of Vitek 2 AST-GN69 and AST-XN06 cards were compared to Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) for 99 isolates of *Pseudomonas aeruginosa*, 26 *Acinetobacter baumannii* isolates, and 11 *Stenotrophomonas maltophilia* isolates. In total, 15 antimicrobials were evaluated, with 11 for *P. aeruginosa*, 14 for *A. baumannii*, and 2 for *S. maltophilia*. Categorical agreement (CA) was assessed using both Vitek 2 breakpoints and 2016 CLSI M100S 26th edition breakpoints. The essential agreement values for *P. aeruginosa*, *A. baumannii*, and *S. maltophilia* were 99.5%, 99.2%, and 100%, respectively. The CA values for *P. aeruginosa*, *A. baumannii*, and *S. maltophilia* were 94.1%, 92.7%, and 95.5%, respectively, by the Vitek 2 breakpoints, and 93.4%, 92.3%, and 95.5%, respectively, by the CLSI breakpoints. Overall, the Vitek 2 performance was comparable to that of BMD using both Vitek 2 breakpoints and 2016 CLSI M100S 26th edition breakpoints. Improved performance was noted for the reformulated piperacillin-tazobactam and imipenem found on the AST-GN69 card, with no very major or major errors noted when using the CLSI breakpoints.

KEYWORDS *Acinetobacter baumannii*, antimicrobial susceptibility testing, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, Vitek 2, breakpoints, broth microdilution

Multidrug resistance (MDR) among Gram-negative bacteria, which is defined by nonsusceptibility (intermediate or resistant) to ≥1 agent in ≥3 antimicrobial categories (1), is a significant clinical concern. In the United States, 12.6% of health care-associated infections (HAIs) caused by *Pseudomonas aeruginosa* are due to MDR isolates, as are 45.3% of *Acinetobacter* species (2). Infections caused by these MDR organisms are associated with poor clinical outcomes, particularly for patients who are immunocompromised, have prolonged hospitalization in the intensive care unit, or who reside in long-term-care facilities (3). The treatment options for MDR infections are limited, making accurate and timely antimicrobial susceptibility testing (AST) critical for patient care.

Most U.S. clinical laboratories use commercial automated systems for AST, including the bioMérieux Vitek 2. However, the failure of these systems to detect resistance in Gram-negative bacteria, and in particular β-lactam resistance in nonfermenting Gram-negative bacilli, such as *P. aeruginosa* and *Acinetobacter baumannii*, has been reported by several studies (4–7). In 2010, bioMérieux issued a voluntary recall of AST cards

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TABLE 1 Overall performance of AST-GN69 and AST-NX06 cards compared to BMD for 91 *P. aeruginosa*, 26 *A. baumannii*, and 11 *S. maltophilia* isolates

			Performance measure[c]				
Organism group	BP[a]	Total[b]	EA (%)	CA (%)	VMEs (no. [%])	MEs (no. [%])	mEs (no. [%])
P. aeruginosa	V2	1,001	99.5	94.1	0 (0)	12 (1.9)	51 (4.6)
A. baumannii	V2	364	99.2	92.7	1 (7.1)	0 (0)	31 (5.2)
S. maltophilia	V2	22	100	95.5	0 (0)	0 (0)	1 (4.6)
P. aeruginosa	CLSI	1,001	99.5	93.4	0 (0)	1 (0.12)	67 (6.5)
A. baumannii	CLSI	364	99.2	92.3	1 (7.1)	0 (0)	33 (5.3)
S. maltophilia	CLSI	22	100	95.5	0 (0)	0 (0)	1 (4.6)

[a]BP, breakpoint used to interpret MIC results; V2, Vitek 2 breakpoints; CLSI, M100S 26th edition breakpoints.
[b]Total represents the number of isolates tested multiplied by the number of antimicrobials tested.
[c]EA, essential agreement (MIC ±1 doubling dilution); CA, categorical agreement; VMEs, very major errors; MEs, major errors; mEs, minor errors.

containing piperacillin-tazobactam, and they reformulated piperacillin-tazobactam and imipenem on Vitek 2 cards and revised Vitek 2 software to correct these problems in 2012; however, only one published study has independently evaluated the performance of these changes, and this was for the *Enterobacteriaceae* (8). Additionally, both the Clinical and Laboratory Standards Institute (CLSI) and the U.S. Food and Drug Administration (FDA) have revised breakpoints for several agents commonly tested against Gram-negative bacteria. These changes include revision of the *P. aeruginosa* breakpoints for imipenem, meropenem, and piperacillin-tazobactam, and revision of the *Acinetobacter* species breakpoints for imipenem and meropenem by one or both of these organizations. CLSI added doripenem breakpoints in 2009, but these remain different from the current FDA doripenem breakpoints. At present, Vitek 2 is only FDA cleared for use with historical *P. aeruginosa* and *Acinetobacter* species breakpoints (i.e., 2009 FDA breakpoints, which are the same as those published in the 2009 CLSI M100-S19 standard [9]). Laboratories that use this system out of the box thus report MICs using 2009 FDA carbapenem and piperacillin-tazobactam breakpoints for these organisms, which differ from current CLSI and FDA breakpoints (9). Herein, we refer to these historical breakpoints as "Vitek 2 breakpoints." Clinical laboratories can manually revise breakpoints applied to MICs obtained on their commercial AST systems, but only after the performance of the system with the revised breakpoints has been verified by the laboratory. Few laboratories have the resources to perform these studies, and limited published data document the performance of commercial systems used off-label with revised breakpoints.

We evaluated the currently available Vitek 2 AST-GN69 (containing reformulated piperacillin-tazobactam and imipenem, released in 2012) and AST-XN06 cards compared to a CLSI reference broth microdilution (BMD) method. Fifteen antimicrobials (11 on AST-GN69 and 4 on AST-XN06) were tested between the two cards using contemporary bacterial isolates.

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RESULTS

Of 99 *P. aeruginosa* isolates tested, 8 isolates (8.1%) were eliminated due to repeated growth control failures on the Vitek 2. When MIC results were interpreted using Vitek 2 breakpoints for the remaining 91 *P. aeruginosa* isolates, initial testing revealed 16 major errors (MEs), 4 of which resolved by repeat testing, yielding an overall 99.5% essential agreement (EA), 94.1% categorical agreement (CA), and 12 MEs and 51 minor errors (mEs) (Table 1). Three MEs were for piperacillin-tazobactam-susceptible isolates that tested resistant by Vitek 2 and susceptible by BMD (Table 2). All 3 isolates had a BMD MIC of 64 μg/ml and Vitek 2 MIC of $\geq$128 μg/ml. The remaining 9 MEs were for doripenem (Table 2). Six of 9 MEs were in isolates with a BMD MIC of 2 μg/ml and Vitek 2 MIC of 4 μg/ml (i.e., essential agreement), whereas the remaining 3 had BMD MICs $>$1-log$_2$-dilution lower than the Vitek 2 MIC. By M100S 26th edition breakpoints (10),

TABLE 2 Performance of AST-GN69 and AST-NX06 cards compared to BMD for 91 *P. aeruginosa* isolates

		No. of isolates[c]				Performance (no. [%])[d]				
Antimicrobial[a]	BP[b]	Total	R	I	S	EA	CA	VMEs	MEs	mEs
TZP	V2	91	21	0	70	90 (98.9)	88 (96.7)	0 (0)	3 (4.3)	0 (0)
	CLSI	91	21	11	59	90 (98.9)	86 (94.5)	0 (0)	0 (0)	5 (5.5)
Cefepime	V2/C	91	14	14	63	91 (100)	81 (89.0)	0 (0)	0 (0)	10 (11.0)
Ceftazidime	V2/C	91	16	9	66	91 (100)	83 (91.2)	0 (0)	0 (0)	8 (8.8)
Doripenem	V2	91	35	0	56	88 (96.7)	82 (90.1)	0 (0)	9 (16.1)	0 (0)
	CLSI	91	22	13	56	88 (96.7)	70 (76.9)	0 (0)	1 (1.8)	20 (22.0)
Imipenem	V2	91	31	6	54	91 (100)	84 (92.3)	0 (0)	0 (0)	7 (7.7)
	CLSI	91	37	4	50	91 (100)	88 (96.7)	0 (0)	0 (0)	3 (3.3)
Meropenem	V2	91	25	10	56	91 (100)	84 (92.3)	0 (0)	0 (0)	7 (7.7)
	CLSI	91	34	5	52	91 (100)	89 (97.8)	0 (0)	0 (0)	2 (2.2)
Amikacin	V2/C	91	2	2	87	91 (100)	91 (100)	0 (0)	0 (0)	0 (0)
Gentamicin	V2/C	91	10	0	81	90 (98.9)	79 (86.8)	0 (0)	0 (0)	12 (13.2)
Tobramycin	V2/C	91	8	0	83	91 (100)	90 (98.9)	0 (0)	0 (0)	1 (1.1)
Ciprofloxacin	V2/C	91	23	5	63	91 (100)	88 (96.7)	0 (0)	0 (0)	3 (3.3)
Levofloxacin	V2/C	91	27	10	54	91 (100)	88 (96.7)	0 (0)	0 (0)	3 (3.3)

[a]TZP, piperacillin-tazobactam.
[b]BP, breakpoint used to interpret MIC results; V2/C, Vitek 2 and CLSI M100S 26th edition breakpoints are the same; V2, Vitek 2 reported breakpoints; CLSI, M100S 26th edition breakpoints.
[c]R, resistant; I, intermediate; S, susceptible.
[d]EA, essential agreement (MIC ±1 doubling dilution); CA, categorical agreement; VMEs, very major errors; MEs, major errors; mEs, minor errors.

initial testing revealed 3 MEs. Two MEs corrected upon repeat testing, yielding an overall 93.4% CA, 1 MEs, and 67 mEs (Table 1). All 3 piperacillin-tazobactam MEs observed by the Vitek 2 breakpoints were corrected by use of the CLSI M100S 26th edition breakpoints, and 8 of 9 doripenem MEs changed to mEs. However, a significant increase in doripenem mEs was observed when applying the M100S 26th edition breakpoints due to the creation of intermediate and resistant breakpoints. Twenty doripenem mEs (22%) were observed (Table 2), all for isolates with a Vitek 2 MIC that was 1-$\log_2$-dilution higher than the BMD MIC.

For *A. baumannii*, no growth terminations were observed among the 26 isolates tested. Initial testing revealed 6 VMEs and 1 ME by both Vitek 2 and M100S 26th edition breakpoints. Repeat testing corrected 5 of 6 VMEs and the ME; in all cases, the initial BMD result was the source of the error. Overall, 99.2% EA, 92.7% CA, 1 very major error (VME), and 31 mEs were observed using the Vitek 2 breakpoints (Table 1). By CLSI M100S 26th edition breakpoints, there was 1 VME and 33 mEs, resulting in a CA of 92.3% (Table 1). The 1 VME by both Vitek 2 and CLSI M100S 26th edition breakpoints was for tobramycin in a meropenem-resistant *A. baumannii* isolate (Table 3). mEs were observed by both Vitek 2 and M100S 26th edition breakpoints in the β-lactam–β-lactamase inhibitor combinations and the cephems (Table 3). The Vitek 2 MIC was 1-$\log_2$ dilution above the BMD MIC for these mEs in all cases, except for ampicillin-sulbactam, where the Vitek 2 MIC was 1-$\log_2$ dilution below the BMD MIC.

Among 11 *Stenotrophomonas maltophilia* isolates evaluated, no growth terminations, VMEs, or MEs were observed, resulting in 100% EA, 95.5% CA, and 1 mE by both Vitek 2 and M100S 26th edition breakpoints (Table 1). The sole *S. maltophilia* error was an mE for a levofloxacin-resistant isolate that tested 1-$\log_2$ dilution below the BMD MIC by Vitek 2 (Table 4).

DISCUSSION

Vitek 2 performed well for AST of *P. aeruginosa*, *A. baumannii*, and *S. maltophilia* isolates evaluated in this study. The CA using the CLSI M100S 26th edition breakpoints

TABLE 3 Performance of AST-GN69 and AST-NX06 cards compared to BMD for 26 *A. baumannii* isolates

		No. of isolates[c]				Performance (no. [%])[d]				
Antimicrobial[a]	BP[b]	Total	R	I	S	EA	CA	VMEs	MEs	mEs
SAM	V2/C	26	7	6	13	26 (100)	18 (69.2)	0 (0)	0 (0)	8 (30.7)
TZP	V2/C	26	13	2	11	26 (100)	23 (88.5)	0 (0)	0 (0)	3 (11.5)
Cefepime	V2/C	26	10	4	12	26 (100)	21 (80.8)	0 (0)	0 (0)	5 (19.2)
Cefotaxime	V2/C	26	14	8	6	26 (100)	23 (88.5)	0 (0)	0 (0)	3 (11.5)
Ceftazidime	V2/C	26	13	1	12	25 (96.2)	24 (85.7)	0 (0)	0 (0)	4 (15.4)
Ceftriaxone	V2/C	26	13	7	6	25 (96.2)	20 (76.9)	0 (0)	0 (0)	6 (23.1)
Doripenem	V2	26	13	0	13	26 (100)	26 (100)	0 (0)	0 (0)	0 (0)
	CLSI	26	14	0	12	26 (100)	25 (96.2)	0 (0)	0 (0)	1 (3.8)
Imipenem	V2	26	10	0	16	26 (100)	26 (100)	0 (0)	0 (0)	0 (0)
	CLSI	26	10	1	15	26 (100)	26 (100)	0 (0)	0 (0)	0 (0)
Meropenem	V2	26	10	1	15	26 (100)	26 (100)	0 (0)	0 (0)	0 (0)
	CLSI	26	11	1	14	26 (100)	25 (96.2)	0 (0)	0 (0)	1 (3.8)
Gentamicin	V2/C	26	11	0	15	26 (100)	26 (100)	0 (0)	0 (0)	0 (0)
Tobramycin	V2/C	26	10	0	16	25 (96.2)	23 (88.5)	1 (10.0)	0 (0)	2 (7.7)
Ciprofloxacin	V2/C	26	14	0	12	26 (100)	26 (100)	0 (0)	0 (0)	0 (0)
Levofloxacin	V2/C	26	14	0	12	26 (100)	26 (100)	0 (0)	0 (0)	0 (0)
SXT	V2/C	26	13	0	13	26 (100)	26 (100)	0 (0)	0 (0)	0 (0)

[a]SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; SXT, trimethoprim-sulfamethoxazole.
[b]BP, breakpoint used to interpret MIC results; V2/C, Vitek 2 and CLSI M100S 26th edition breakpoints are the same; V2, Vitek 2 reported breakpoints; CLSI, M100S 26th edition breakpoints.
[c]R, resistant; I, intermediate; S, susceptible.
[d]EA, essential agreement (MIC ±1 doubling dilution); CA, categorical agreement; VMEs, very major errors; MEs, major errors; mEs, minor errors.

was slightly lower than that with the Vitek 2 breakpoints. This can be attributed to the creation of intermediate breakpoints for piperacillin-tazobactam and doripenem, which resulted in a higher number of mEs. Overall performance was acceptable, with EA and CA of ≥90% and run failures of <10%. The 8 *P. aeruginosa* isolates that had repeat growth control failures were all mucoid strains, a known limitation of automated AST systems. Due to this problem, laboratories might consider primary testing of mucoid isolates by disk diffusion. The most notable errors occurred in *P. aeruginosa* isolates with piperacillin-tazobactam, as has been seen by others (5, 7, 11, 12). Unlike these previous studies that found piperacillin-tazobactam VME rates ranging from 10.2 to 21.7% (5, 7, 10), we found 0 VMEs and only 3 (4.3%) MEs. This improved performance may be attributable to the reformulation of piperacillin-tazobactam on the AST-GN69 cards. These 3 MEs by Vitek 2 breakpoints were for isolates with Vitek 2 MICs one dilution higher than the BMD MIC. By applying the CLSI M100S 26th edition breakpoints, these 3 MEs became mEs. It should be noted that the package insert for Vitek 2 includes a limitation for AST-GN69, requiring performance of an alternative method before reporting a resistant result for piperacillin-tazobactam and *P. aeruginosa* (13),

TABLE 4 Performance of AST-GN69 and AST-NX06 cards compared to BMD for 11 *S. maltophilia* isolates

		No. of isolates[c]				Performance (no. [%])[d]				
Antimicrobial[a]	BP[b]	Total	R	I	S	EA	CA	VMEs	MEs	mEs
Levofloxacin	V2/C	11	1	0	10	11 (100)	10 (90.9)	0 (0)	0 (0)	1 (9.1)
SXT	V2/C	11	2	0	9	11 (100)	11 (100)	0 (0)	0 (0)	0 (0)

[a]SXT, trimethoprim-sulfamethoxazole.
[b]BP, breakpoint used to interpret MIC results; V2/C, Vitek 2 and CLSI M100S 26th edition breakpoints are the same.
[c]R, resistant; I, intermediate; S, susceptible.
[d]EA, essential agreement (MIC ±1 doubling dilution); CA, categorical agreement; VMEs, very major errors; MEs, major errors; mEs, minor errors.

although we did not note any significant discrepancies for this drug-organism combination in this study.

Carbapenem resistance, which is an increasingly common occurrence among clinical isolates of *P. aeruginosa* and *A. baumannii*, was detected by Vitek 2 using both the M100S 26th edition and Vitek 2 breakpoints. One exception is doripenem and *P. aeruginosa*, with 16.1% MEs using Vitek 2 breakpoints and 22% mEs using M100S 26th edition breakpoints. Vitek 2 could still be used for doripenem testing, with the knowledge that isolates with intermediate results by the CLSI M100S 26th edition breakpoints may in fact be susceptible, and alternative testing should be done to confirm susceptibility for these isolates.

The limitations of our study include the small number of *A. baumannii* and *S. maltophilia* isolates tested, ultimately leading to a >10% mE rate for 6 of the 14 antimicrobials tested for *A. baumannii* and an overall CA of 92.7%. The high percentage of mEs can be misleading because of the small number of isolates tested, and caution should be used when interpreting the performance of Vitek 2 for this organism with beta-lactamase inhibitor combinations and cephems. Particular attention should be paid to ampicillin-sulbactam, which consistently showed a Vitek 2 trend toward false-susceptible results. Further studies with additional contemporary isolates of *A. baumannii* and *S. maltophilia* are needed. Of note, bioMérieux has yet to seek FDA clearance for the updated *Acinetobacter* species breakpoints. However, it is important to note that the FDA will only clear susceptibility tests for organisms that are specifically listed as clinically indicated for a given antimicrobial in the prescribing information (14). As such, if bioMérieux were to attempt to obtain FDA clearance of updated CLSI/FDA breakpoints for *Acinetobacter* spp., meropenem would not be cleared by the FDA, leaving laboratories with fewer testing options for this organism than are currently available on the system cleared with historical breakpoints.

In summary, Vitek 2 performance was satisfactory, compared to BMD, for a collection of contemporary isolates of *P. aeruginosa*, *A. baumannii*, and *S. maltophilia*. For laboratories that do not routinely use Vitek 2 for nonfermenting Gram-negative bacilli, the previously reported issues with piperacillin-tazobactam and imipenem have appeared to be resolved with the reformulated AST card and upgraded software. Based on our data, we recommend that laboratories play close attention to MICs close to the breakpoints and monitor the performance of their test system against a reference standard.

MATERIALS AND METHODS

Bacterial isolates. Ninety-nine *P. aeruginosa* isolates, 26 *A. baumannii* isolates, and 11 *Stenotrophomonas maltophilia* isolates were included in this study. These isolates were recovered from clinical cultures between 2012 and 2013 at our institution. The isolates were selected to represent a variety of resistance phenotypes that cover a wide range of MICs.

Prior to testing, frozen isolates were subcultured twice, and fresh isolates were subcultured once on tryptic soy agar plates containing 5% sheep blood (BAP; BD, Sparks, MD). Quality control (QC) strains tested with each run included *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Upon receipt of a new shipment or lot of Vitek 2 cards, the following QC strains were tested: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *E. coli* ATCC 35218. *K. pneumoniae* ATCC 700603 was also tested on AST-GN69 only.

Antimicrobial susceptibility testing. Each isolate was tested concurrently by the two methods using 3 to 5 isolated colonies from a single 18- to 24-h BAP. BMD MIC testing was performed according to CLSI standards, using panels prepared in-house (15). Panels were incubated at 35°C in ambient air and read manually following 16 to 20 h of incubation. Vitek 2 (bioMérieux, Inc., Durham, NC, USA) testing was performed using software version 5.04 and AST-GN69 and AST-XN06 cards, according to the manufacturer's instructions (13, 16).

Data analysis. Calculations of essential agreement (EA), categorical agreement (CA), very major errors (VMEs), major errors (MEs), and minor errors (mEs) were done as previously described (17). The Vitek 2 MICs were compared to the reference BMD MICs. EA was defined as an MIC ±1 doubling dilution of the reference BMD MIC. The CA was defined as a susceptible, intermediate, resistant, or nonsusceptible result that was the same with the two methods. A VME was defined as a false-susceptible result with Vitek 2, whereas an ME was a false-resistant or nonsusceptible result. An mE was defined as an intermediate result with one method and a susceptible or resistant result with other method. All MICs (obtained by Vitek 2 and BMD) were evaluated two ways: (i) by Vitek 2 breakpoints (applied by the

TABLE 5 Vitek 2 and CLSI M100S 26th edition breakpoints that differ for *P. aeruginosa* and *A. baumannii*

		MIC (μg/ml) by organism					
		P. aeruginosa[c]			*A. baumannii*		
Antimicrobial[a]	BP[b]	S	I	R	S	I	R
Doripenem	V2	≤2			≤1		
	CLSI	≤2	4	≥8	≤2	4	≥8
Imipenem	V2	≤4	8	≥16	≤4	8	≥16
	CLSI	≤2	4	≥8	≤2	4	≥8
Meropenem	V2	≤4	8	≥16	≤4	8	≥16
	CLSI	≤2	4	≥8	≤2	4	≥8
TZP	V2	≤64/4		≥128/4			
	CLSI	≤16/4	32/4–64/4	≥128/4			

[a]TZP, piperacillin-tazobactam.
[b]BP, breakpoint used to interpret MIC results; V2, Vitek 2 reported breakpoints; CLSI, M100S 26th edition breakpoints.
[c]R, resistant; I, intermediate; S, susceptible.

Vitek 2 system software version 5.04) and (ii) by CLSI M100S 26th edition breakpoints (10). The Vitek 2 and CLSI M100S 26th edition breakpoints differ for doripenem, imipenem, and meropenem for both *P. aeruginosa* and *A. baumannii*, and for piperacillin-tazobactam and *P. aeruginosa*. These breakpoints are listed in Table 5.

Discrepant resolution. Isolates with a VME or ME were retested in parallel using both methods, as were isolates with growth control failures on Vitek 2 cards. EA, CA, VMEs, MEs, and mEs were calculated after repeat testing. If an error persisted after repeat testing, it was included in the calculations. If an error resolved after repeat testing, it was not counted as an error, and the initial result was disregarded. Isolates that terminated due to failed growth after repeat testing were excluded from the analysis.

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