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Comparison of the Vitek 2, MicroScan, and Etest Methods with the Agar Dilution Method in Assessing Colistin Susceptibility of Bloodstream Isolates of *Acinetobacter* Species from a Korean University Hospital

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We evaluated three commercial colistin susceptibility testing methods using 213 bloodstream *Acinetobacter* isolates identified by gene sequencing. Compared to the agar dilution reference method, excellent categorical agreements (both 99.1%) were observed using Vitek 2 and Etest, compared to 87.3% (95.7% for *Acinetobacter baumannii* and 80.7% for non-*baumannii Acinetobacter* isolates) using MicroScan.

Acinetobacter species have emerged as important causative pathogens of a variety of nosocomial infections such as bacteremia, hospital-acquired pneumonia, and urinary tract infections (1). This organism commonly presents resistance to multiple antimicrobial agents, including carbapenems (multidrug resistance), except to an "old" drug, colistin (polymyxin E), which often remains the only effective therapeutic option (2). However, *Acinetobacter* strains developing resistance to colistin have recently been described (3). Therefore, rapid and reliable methods for colistin susceptibility testing of *Acinetobacter* species are needed (4).

High error rates and low levels of reproducibility of the disk diffusion method for detecting colistin resistance are well documented (4), but Etest has shown excellent agreement with agar dilution (AD) and broth microdilution for testing colistin resistance in major Gram-negative bacteria (4–6). However, the performance of automated antimicrobial susceptibility testing systems has rarely been assessed in terms of determining colistin susceptibility in *Acinetobacter* species, especially for non-*baumannii Acinetobacter* strains (4, 7). In this study, by testing a broad range of *Acinetobacter* species isolated from blood cultures at a university hospital during a 4-year period, we evaluated the suitability of three commercial methods for colistin susceptibility testing—Vitek 2 (Vitek 2 XL; bioMérieux, Hazelwood, MO), MicroScan (MicroScan WalkAway 96 Plus; Siemens Healthcare Diagnostics Inc., Deerfield, IL), and Etest (Etest; bioMérieux SA, Marcy l'Etoile, France)—in comparison with the AD reference method.

A total of 213 nonduplicate bloodstream infection (BSI) isolates of *Acinetobacter* species were obtained from patient blood cultures at Chonnam National University Hospital (a 1,000-bed tertiary care hospital in Gwangju, Republic of Korea) from January 2008 to December 2011. Isolates were identified by partial *rpoB* gene sequencing (8). DNA extraction and sequencing were performed as described previously (9). Colistin susceptibility testing with the Etest (Colistin CO 256), Vitek 2 (Gram-negative susceptibility card AST-N132), and MicroScan (Gram-negative breakpoint combo panel type 42) methods was performed according to the manufacturers' instructions. The AD method was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (10, 11). Categorical results of each method were analyzed on the basis of the CLSI breakpoint for colistin (MIC of ≤2 μg/ml, susceptible [S], and MIC of ≥4 μg/ml, resistant) (11). Categorical agreement (CA) was defined as the percentage of isolates classified into the same category by the AD reference method, and very major error (VME) and major error (ME) were defined as described previously (12).

Of all 213 *Acinetobacter* BSI isolates, 13 (6.1%) demonstrated colistin resistance (MIC of ≥4 μg/ml) by the AD method, which included 10 isolates of *Acinetobacter* genomic species 13BJ (GS13BJ), two isolates of *A. junii*, and one isolate of *A. nosocomialis*. Overall, 100% (94/94) isolates of *A. baumannii* were susceptible to colistin, while 10.9% (13/119) isolates of non-*baumannii Acinetobacter* species were resistant. This finding supports previous reports showing that resistance to colistin may be more common in non-*baumannii Acinetobacter* isolates than in *A. baumannii* (13). For all 213 isolates, excellent CA (both ≥99%) with AD was observed with Vitek 2 and Etest, but CA with AD using MicroScan was 87.3% (Table 1).

To date, only two studies have investigated the performance of Vitek 2 for colistin susceptibility testing using clinical isolates of *Acinetobacter* species (4, 7). However, as only 2 of the 67 isolates in these studies were colistin resistant, it is not possible to draw definitive conclusions regarding the efficacy of the Vitek 2 system for detection of colistin resistance in *Acinetobacter* species until more resistant isolates are tested (4, 7). In this study, we tested 213 BSI

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TABLE 1 Distribution of colistin MICs as determined by the agar dilution reference method, Vitek 2, MicroScan, and Etest and categorical agreement between results obtained by the agar dilution method and each of the other three methods for 213 *Acinetobacter* bloodstream isolates

Species (no. of isolates)	Test system[a]	No. of isolates with colistin MIC (μg/ml):								No. (%) of isolates with result:		% categorical agreement with the agar dilution method	% of category errors	
		≤0.5	1	2	4	8	16	32	>32	Susceptible	Resistant		Very major	Major
A. baumannii (94)	Agar dilution	28	49	17	0	0	0	0	0	94 (100)	0 (0)			
	Vitek 2	79	11	4	0	0	0			94 (100)	0 (0)	100	0	0
	MicroScan			90	3	1				90 (95.7)	4 (4.3)	95.7	0	4.3
	Etest	93	0	0	0	0	0	1	0	93 (98.9)	1 (1.1)	98.9	0	1.1
A. nosocomialis (67)	Agar dilution	6	42	18	1	0	0	0	0	66 (98.5)	1 (1.5)			
	Vitek 2	40	16	11	0	0	0			67 (100)	0 (0)	98.5	1.5	0
	MicroScan			52	13	2				52 (77.6)	15 (22.4)	79.1	0	20.9
	Etest	64	2	0	0	0	0	1	0	66 (98.5)	1 (1.5)	100	0	0
A. pittii (12)	Agar dilution	5	6	1	0	0	0	0	0	12 (100)	0 (0)			
	Vitek 2	12	0	0	0	0	0			12 (100)	0 (0)	100	0	0
	MicroScan			12	0	0				12 (100)	0 (0)	100	0	0
	Etest	11	0	0	0	0	0	1	0	11 (91.7)	1 (8.3)	91.7	0	8.3
Acinetobacter genomic species 13BJ (10)	Agar dilution	0	0	0	0	1	2	3	4	0 (0)	10 (100)			
	Vitek 2	0	0	1	2	0	7			1 (10.0)	9 (90.0)	90.0	10.0	0
	MicroScan			2	0	8				2 (20.0)	8 (80.0)	80.0	20.0	0
	Etest	0	0	0	1	0	2	7	0	0 (0)	10 (100)	100	0	0
A. ursingii (7)	Agar dilution	5	2	0	0	0	0	0	0	7 (100)	0 (0)			
	Vitek 2	7	0	0	0	0	0			7 (100)	0 (0)	100	0	0
	MicroScan			7	0	0				7 (100)	0 (0)	100	0	0
	Etest	7	0	0	0	0	0	0	0	7 (100)	0 (0)	100	0	0
A. soli (6)	Agar dilution	0	4	2	0	0	0	0	0	6 (100)	0 (0)			
	Vitek 2	6	0	0	0	0	0			6 (100)	0 (0)	100	0	0
	MicroScan			6	0	0				6 (100)	0 (0)	100	0	0
	Etest	3	3	0	0	0	0	0	0	6 (100)	0 (0)	100	0	0
A. bereziniae (5)	Agar dilution	0	2	3	0	0	0	0	0	5 (100)	0 (0)			
	Vitek 2	0	3	2	0	0	0			5 (100)	0 (0)	100	0	0
	MicroScan			0	1	4				0 (0)	5 (100)	0	0	100
	Etest	5	0	0	0	0	0	0	0	5 (100)	0 (0)	100	0	0
A. junii (3)	Agar dilution	0	1	0	0	1	1	0	0	1 (33.3)	2 (66.7)			
	Vitek 2	0	1	0	0	1	1			1 (33.3)	2 (66.7)	100	0	0
	MicroScan			1	1	1				1 (33.3)	2 (66.7)	100	0	0
	Etest	0	1	0	0	2	0	0	0	1 (33.3)	2 (66.7)	100	0	0
A. johnsonii (1)	Agar dilution	1	0	0	0	0	0	0	0	1 (100)	0 (0)			
	Vitek 2	1	0	0	0	0	0			1 (100)	0 (0)	100	0	0
	MicroScan			1	0	0				1 (100)	0 (0)	100	0	0
	Etest	0	1	0	0	0	0	0	0	1 (100)	0 (0)	100	0	0
Other *Acinetobacter* species (8)	Agar dilution	0	3	5	0	0	0	0	0	8 (100)	0 (0)			
	Vitek 2	4	2	2	0	0	0			8 (100)	0 (0)	100	0	0
	MicroScan			6	1	1				6 (75.0)	2 (25.0)	75.0	0	25.0
	Etest	8	0	0	0	0	0	0	0	8 (100)	0 (0)	100	0	0
Total (213)	Agar dilution	45	109	46	1	2	3	3	4	200 (93.9)	13 (6.1)			
	Vitek 2	149	33	20	2	1	8			202 (94.8)	11 (5.2)	99.1	0.9	0
	MicroScan			177	19	17				177 (83.1)	36 (16.9)	87.3	0.9	11.7
	Etest	191	7	0	1	2	2	10	0	198 (93.0)	15 (7.0)	99.1	0	0.9

[a] The colistin MIC ranges tested were ≤0.5 to >32 μg/ml by the agar dilution method. The colistin MIC ranges determined by Vitek 2 were ≤0.5 to ≥16 μg/ml, and MICs of ≥16 μg/ml determined by Vitek 2 were presented as 16 μg/ml. The colistin MIC ranges determined by MicroScan were ≤2 to >4 μg/ml, and MICs of ≤2 and >4 μg/ml determined by MicroScan were presented as 2 and 8 μg/ml, respectively. Etest MICs were rounded up to the next 2-fold dilution value.

isolates, including 13 isolates of colistin-resistant *Acinetobacter* species. Vitek 2 showed excellent CA with 0.9% VMEs and no ME.

Etest also showed excellent CA with AD for both *A. baumannii* (98.9%) and non-*baumannii Acinetobacter* species (99.2%). Although colistin Etest gives high error rates for cystic fibrosis isolates of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* (14), our data showed no VME and only 0.9% MEs of the colistin Etest for *Acinetobacter* species, results which are in agreement with other reports (4, 7).

The performance of MicroScan for colistin susceptibility testing has not been reported. In our study, the CA with AD for MicroScan was 95.7% for *A. baumannii* but 80.7% for non-*baumannii Acinetobacter* isolates. MicroScan produced VMEs in two isolates of *Acinetobacter* GS13BJ and MEs in 25 isolates, composed of 4.3% (4/94) *A. baumannii* and 17.6% (21/119) non-*baumannii Acinetobacter* species, suggesting that non-*baumannii Acinetobacter* species rather than *A. baumannii* were the main source of errors. Reasons for the low CA with MicroScan are unknown. However, MicroScan generated the narrowest distribution of colistin MICs ($\leq$2 to $>$4 μg/ml), compared with Etest (0.016 to 256 μg/ml) or Vitek 2 ($\leq$0.5 to $\geq$16 μg/ml). Considering the CLSI breakpoint for colistin (susceptible [S], $\leq$2 μg/ml; resistant $\geq$ 4 μg/ml), the presence of only three point ($\leq$2, 4, and $>$4 μg/ml) determinations of colistin MICs in the MicroScan system appears to create difficulty in differentiating colistin-resistant from -susceptible *Acinetobacter* isolates.

The limitation of our study is that no colistin-resistant *A. baumannii* isolates were tested. However, our study included 10 isolates of *Acinetobacter* GS13BJ, which has consistently been reported to be resistant to colistin (1, 13, 15). In the present study, of 10 *Acinetobacter* GS13BJ isolates, 10 (100%), 9 (90%), 8 (80%), and 10 (100%) had a colistin MIC of $\geq$4 μg/ml by AD, Vitek 2, MicroScan, and Etest, respectively. These data suggest that *Acinetobacter* GS13BJ is innately resistant to colistin and that the AD and Etest, followed by Vitek 2, have the best ability to detect colistin resistance in this species.

In conclusion, our findings indicate, for the first time, that the MicroScan is unsuitable for colistin susceptibility testing of *Acinetobacter* species, especially non-*baumannii Acinetobacter* species, due to its low reliability; in contrast, Etest and Vitek 2 are useful methods for discrimination of colistin-resistant and -susceptible *Acinetobacter* isolates.

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