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Comparative Evaluation of Tigecycline Susceptibility Testing Methods for Expanded-Spectrum Cephalosporin- and Carbapenem-Resistant Gram-Negative Pathogens

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We evaluated the Vitek2, Etest, and MIC Test Strip (MTS) methods of tigecycline susceptibility testing with 241 expanded-spectrum cephalosporin-resistant and/or carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* clinical isolates by using dry-form broth microdilution (BMD) as the reference method. The $MIC_{50/90}$s were as follows: BMD, 1/4 μg/ml; Vitek2, 4/≥8 μg/ml; Etest, 2/4 μg/ml; MTS, 0.5/2 μg/ml. Vitek2 produced 9.1/21.2% major errors, Etest produced 0.4/0.8% major errors, and MTS produced no major errors but 0.4/3.3% very major errors (FDA/EUCAST breakpoints). Vitek2 tigecycline results require confirmation by BMD or Etest for multidrug-resistant pathogens.

Carbapenem resistance has steadily increased in several regions, representing the most significant resistance issue among multidrug-resistant (MDR) Gram-negative pathogens (2, 17, 18). Tigecycline and colistin are among the few antimicrobials active and are commonly used for infections caused by carbapenem-resistant (CR) *Enterobacteriaceae* and *Acinetobacter baumannii* (2, 4, 16, 21).

The increasing clinical use of tigecycline necessitates rapid, simple, and accurate susceptibility testing methods. Several methods have been evaluated for routine tigecycline susceptibility testing (1, 12, 14, 19, 22) with broth microdilution (BMD) as the reference method. It has been previously noted that discrepancies may exist when different methods are used (3, 7, 14, 19, 22). It was also reported that disk diffusion and Etest have a poor correlation with BMD, especially for *A. baumannii* (3, 10, 12, 22). Furthermore, the accuracy of the Vitek2 automated system (bioMérieux, Marcy l' Etoile, France) has not been adequately evaluated for tigecycline (15).

In this study, we evaluated three routine tigecycline susceptibility methods, Vitek2, Etest (bioMérieux), and MIC Test Strip (MTS; Liofilchem SRL, Roseto degli Abruzzi, Italy) in comparison with BMD. These methods were applied to a large collection of CR *Enterobacteriaceae* and *A. baumannii* and expanded-spectrum cephalosporin-resistant (ESCR) *Enterobacteriaceae* isolates.

Bacterial isolates. This study included 241 clinical isolates recovered during 2008 to 2011 from patients in five tertiary-care hospitals located in different regions of Greece, consisting of CR (*K. pneumoniae* carbapenemase [KPC]-producing *K. pneumoniae*, $n = 73$; VIM-producing *K. pneumoniae*, $n = 39$; KPC- and VIM-producing *K. pneumoniae*, $n = 13$; OXA-58-producing *A. baumannii*, $n = 56$) and ESCR isolates (extended-spectrum β-lactamase [ESBL]-producing *Escherichia coli*, $n = 20$; ESBL-producing *K. pneumoniae*, $n = 20$; *Enterobacter* spp., $n = 20$). Identification was performed with Vitek2. Phenotypic carbapenemase detection was performed as described previously (23). Common broad-spectrum β-lactamase genes (for KPC, OXA-48, OXA-58, metallo-β-lactamases, and ESBLs) were sought by PCR (20).

Susceptibility testing methods. The FDA-cleared commercial dry-form microtiter panels and cation-adjusted Mueller-Hinton broth with *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Sensititre JustOne for tigecycline; TREK Diagnostic Systems, Cleveland, OH) was carried out according to CLSI procedures (6); panels were inoculated manually and read optically, and MICs ranged from 0.06 to 64 μg/ml. The Vitek2 AST-EXN8 susceptibility card containing tigecycline at concentrations of 0.75, 2, and 4 μg/ml was used according to the manufacturer's recommendations. Etest (0.016 to 256 μg/ml) and MTS (0.016 to 256 μg/ml) for tigecycline were performed with Mueller-Hinton II agar (Mast Group Ltd., Bootle, Merseyside, United Kingdom) according to the manufacturer's instructions. Etest and MTS MICs were rounded up to the next 2-fold BMD dilution, as is standard for MIC gradient tests.

All methods were performed simultaneously with a single inoculum of each strain. *E. coli* ATCC 25922 was used as the quality control strain in susceptibility assays.

Definitions and data analysis. There is discordance between the interpretative tigecycline MIC susceptibility breakpoints of *Enterobacteriaceae* issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (susceptible MIC, ≤1 μg/ml; resistant MIC, >2 μg/ml) (8) and the U.S. Food and Drug Administration (FDA) (susceptible MIC, ≤2 μg/ml; resistant MIC, ≥8 μg/ml) (24). For that reason, interpretation of susceptibility results was performed using both the EUCAST and FDA breakpoints. The breakpoints listed for *Enterobacteriaceae* were also applied to *A. baumannii*.

Data were analyzed by comparing the results from each method to those produced by the reference BMD method. MIC_{50}s

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TABLE 1 Tigecycline susceptibilities of the study isolates and MIC_{50}s and MIC_{90}s determined by BMD, Vitek2, Etest, and MTS

Test method and isolate group	No. (%) of isolates						MIC (μg/ml)	
	Susceptible		Intermediate		Resistant			
	FDA	EUCAST	FDA	EUCAST	FDA	EUCAST	50%	90%
BMD								
All isolates	201 (83.4)	150 (62.2)	35 (14.5)	51 (21.2)	5 (2.1)	40 (16.6)	1	4
CR *K. pneumoniae*	105 (84.0)	80 (64.0)	18 (14.4)	25 (20.0)	2 (1.6)	20 (16.0)	1	4
CR *A. baumannii*	42 (75.0)	25 (44.6)	12 (21.4)	17 (30.4)	2 (3.6)	14 (25.0)	2	4
ESCR *Enterobacteriaceae*	54 (90.0)	45 (75.0)	5 (8.3)	9 (15.0)	1 (1.7)	6 (10.0)	0.5	2
Vitek2								
All isolates	103 (42.7)	53 (22.0)	84 (34.9)	50 (20.7)	54 (22.4)	138 (57.3)	4	≥ 8
CR *K. pneumoniae*	50 (40.0)	12 (9.6)	50 (40.0)	38 (30.4)	25 (20.0)	75 (60.0)	4	≥ 8
CR *A. baumannii*	10 (17.9)	3 (5.4)	27 (48.2)	7 (12.5)	19 (33.9)	46 (82.1)	4	≥ 8
ESCR *Enterobacteriaceae*	43 (71.7)	38 (63.3)	7 (11.7)	5 (8.3)	10 (16.7)	17 (28.3)	1	≥ 8
Etest								
All isolates	198 (82.2)	108 (44.8)	33 (13.7)	89 (36.9)	10 (4.1)	44 (18.3)	2	4
CR *K. pneumoniae*	105 (84.0)	48 (38.4)	17 (13.6)	56 (44.8)	3 (2.4)	21 (16.8)	2	4
CR *A. baumannii*	39 (69.6)	16 (28.6)	11 (19.6)	23 (41.1)	6 (10.7)	17 (30.4)	2	4
ESCR *Enterobacteriaceae*	54 (90.0)	44 (73.3)	5 (8.3)	10 (16.7)	1 (1.7)	6 (10.0)	0.5	2
MTS								
All isolates	229 (95.0)	190 (78.8)	9 (3.7)	39 (16.2)	3 (1.2)	12 (5.0)	0.5	2
CR *K. pneumoniae*	124 (99.2)	106 (84.8)	1 (0.8)	18 (14.4)	0 (0)	1 (0.8)	1	2
CR *A. baumannii*	47 (83.9)	32 (57.1)	6 (10.7)	15 (26.8)	3 (5.4)	9 (16.1)	1	4
ESCR *Enterobacteriaceae*	58 (96.7)	52 (86.7)	2 (3.3)	6 (10.0)	0 (0)	2 (3.3)	0.25	2

and MIC_{90}s were calculated. Categorical agreement (CA) was defined as the percentage of isolates classified in the same susceptibility category by BMD and the method under evaluation. Category discrepancies were classified as follows: (i) very major errors (VME), cases where BMD indicated resistance and the comparative method indicated susceptibility; (ii) major errors (ME), an isolate categorized as susceptible by BMD and resistant by the comparative method; (iii) minor errors (mE), one interpretation category difference between BMD and the comparative method (5). Essential agreement (EA) was considered the percentage of MICs within ±1 doubling dilution of the MIC determined by BMD (5).

Acceptable performance was evaluated according to the criteria established by the International Organization for Standardization as follows: ≥90% for EA or CA, ≤3% for VME or ME, and ≤7% for ME plus mE (11).

Susceptibility to tigecycline, EA and CA. The susceptibilities to tigecycline using FDA/EUCAST breakpoints and the MIC_{50} and MIC_{90} for the study isolates determined by each method are presented in Table 1.

By BMD, 201 isolates (83.4%) were tigecycline susceptible using FDA breakpoints while 150 (62.2%) were susceptible using EUCAST breakpoints. CR *A. baumannii* exhibited the lowest susceptibility rates (75/44.6% using FDA/EUCAST breakpoints).

As shown in Table 2, Etest produced susceptibility results similar to those obtained with BMD and CA was high (>80%) for all pathogens using FDA breakpoints. With EUCAST breakpoints, susceptibility rates were lower for most species and CA was 71.8%. Discordant susceptibility rates with serious interpretative errors and rather low CA (48.1/39.4% with FDA/EUCAST breakpoints) were observed for Vitek2. The *in vitro* activity of tigecycline determined by Vitek2 was limited; 57.3/78% of the isolates were classified as either intermediate or resistant. The most pronounced differences in susceptibility rates were noted for CR *A. baumannii* and *K. pneumoniae*. In contrast to Vitek2, a slight shift toward susceptibility was noted for MTS. However, relatively high rates of CA were obtained overall (86.3/68.5%).

BMD, Etest, and MTS resulted in MIC_{50}s of ≤2 μg/ml for all of the species tested, while by Vitek2 the MIC_{50} was 4 μg/ml for CR bacteria. The Etest MIC_{90} was identical to the BMD MIC_{90} for all bacterial groups. MIC_{90}s obtained with Vitek2 (≥8 μg/ml) were inconsistent, compared with those obtained with BMD, while MTS produced lower MIC_{90} values for CR *K. pneumoniae* (Table 1). MICs obtained by MTS were 1, 2, and 3 $\log_2$ dilutions lower for 50.2%, 17.8%, and 2.5% of the isolates, respectively, than those obtained by BMD. In contrast, compared with BMD, Vitek2 resulted in MICs being 1, 2, and 3 $\log_2$ dilutions higher for 41.1%, 36.5%, and 1.7% of the isolates, respectively. By Etest, 47.7% of the isolates exhibited MICs identical to those obtained with BMD and 47.4% of the remaining isolates displayed MICs within ±1 $\log_2$ dilution (Table 3).

EA was highest for Etest (95.0% overall), being ≥90% for all subsets of isolates, exceeding the acceptable performance rate for antimicrobial susceptibility test (AST) methods. MTS resulted in a relatively high rate of EA (77.6%), displaying better performance with CR isolates (EA, 82.4/89.3% for *K. pneumoniae*/*A. baumannii*. On the contrary, Vitek2 generated an overall EA rate of 61.4%, with lower EA rates for CR isolates (52.0 and 55.4%). The EA and CA of MIC results between testing methods compared to the reference BMD are presented in Table 2.

Error classification. Error rates were significantly higher by EUCAST. Etest exhibited the lowest overall error rates (ME, 0.4/

TABLE 2 EA, CA, and types of errors produced when testing tigecycline susceptibility by Vitek2, Etest, and MTS compared to BMD

Test method and isolate group	No. (%) of isolates with:								
		CA		VME		ME		mE	
	EA	FDA	EUCAST	FDA	EUCAST	FDA	EUCAST	FDA	EUCAST
Vitek2									
All isolates	148 (61.4)	116 (48.1)	95 (39.4)	0 (0)	0 (0)	22 (9.1)	51 (21.2)	103 (42.7)	95 (39.4)
CR *K. pneumoniae*	65 (52.0)	57 (45.6)	34 (27.2)	0 (0)	0 (0)	10 (8.0)	32 (25.6)	58 (46.4)	59 (47.2)
CR *A. baumannii*	31 (55.4)	14 (25.0)	18 (32.1)	0 (0)	0 (0)	7 (12.5)	16 (28.6)	35 (62.5)	22 (39.3)
ESCR *Enterobacteriaceae*	52 (86.7)	45 (75.0)	43 (71.7)	0 (0)	0 (0)	5 (8.3)	3 (5.0)	10 (16.7)	14 (23.3)
Etest									
All isolates	229 (95.0)	220 (91.3)	173 (71.8)	0 (0)	0 (0)	1 (0.4)	2 (0.8)	20 (8.3)	66 (27.4)
CR *K. pneumoniae*	121 (96.8)	117 (93.6)	81 (64.8)	0 (0)	0 (0)	1 (0.8)	1 (0.8)	7 (5.6)	43 (34.4)
CR *A. baumannii*	52 (92.9)	47 (83.9)	39 (69.6)	0 (0)	0 (0)	0 (0)	1 (1.8)	9 (16.1)	16 (28.6)
ESCR *Enterobacteriaceae*	56 (93.3)	56 (93.3)	53 (88.3)	0 (0)	0 (0)	0 (0)	0 (0)	4 (6.7)	7 (11.7)
MTS									
All isolates	187 (77.6)	208 (86.3)	165 (68.5)	1 (0.4)	8 (3.3)	0 (0)	0 (0)	32 (13.3)	68 (28.2)
CR *K. pneumoniae*	103 (82.4)	105 (84.0)	80 (64.0)	1 (0.8)	6 (4.8)	0 (0)	0 (0)	19 (15.2)	39 (31.2)
CR *A. baumannii*	50 (89.3)	48 (85.7)	35 (62.5)	0 (0)	1 (1.8)	0 (0)	0 (0)	8 (14.3)	20 (35.7)
ESCR *Enterobacteriaceae*	34 (56.7)	55 (91.7)	50 (83.3)	0 (0)	1 (1.7)	0 (0)	0 (0)	5 (8.3)	9 (15.0)

0.8%; mE, 8.3/27.4%). No MEs were detected for MTS; however, it yielded VMEs (0.4/3.3%) and relatively high mE rates (13.3/28.2%). Vitek2 produced high rates of both MEs (9.1/21.2%) and mEs (42.7/39.4%). With FDA breakpoints, Etest approaches the criteria for acceptable AST performance, while no method displayed acceptable performance with EUCAST breakpoints (Table 2).

The shortage of available treatment options for infections by CR Gram-negative bacteria highlights the importance of accurate tigecycline susceptibility results (9). The interchangeability of tigecycline susceptibility results of CR *Enterobacteriaceae* and *A. baumannii* is not well defined (13). Previous studies have reported discrepant Etest and BMD results with tigecycline, mainly for *A. baumannii* (3, 7, 10, 12, 14, 19, 22). Scarce data are available on the accuracy of VItek2 with MDR pathogens (15). In the present study, we assessed the performance of three routine methods of tigecycline susceptibility testing against ESCR/CR *Enterobacteriaceae* and *A. baumannii* clinical isolates. The results were interpreted by using FDA and EUCAST recommendations.

Of the methods evaluated, Etest showed the best correlation with BMD, exhibiting the lowest error rates and the highest EA and CA. Etest susceptibility results obtained by using FDA criteria meet the AST acceptable-performance criteria for CR *K. pneumoniae* and ESCR *Enterobacteriaceae*, and this could be considered a reliable method for tigecycline testing, as also shown previously (1, 19). A relatively high mE rate of Etest, with slightly decreased susceptibility rates compared to those of BMD was observed for CR *A. baumannii*. In these few cases, Etest generated 1 to 2 $\log_2$ dilution higher MICs. Similar but more pronounced findings have been published previously including higher MICs by Etest for *A. baumannii* (3, 14, 19) and *Enterobacter* spp. (7). Concerns have been expressed regarding the suitability of Etest for tigecycline susceptibility testing against *A. baumannii* (19). With EUCAST breakpoints, a significant increase in mEs was observed, more pronounced for CR *K. pneumoniae*. The elevated mE rates could be explained by the MIC distribution of the organisms' population being close to the breakpoint concentration. The analysis of mEs revealed no trend toward susceptibility or resistance, and thus, error rates were considered acceptable.

We also evaluated MTS for tigecycline susceptibility testing. The level of CA, though lower, was comparable to that of Etest. MTS produced $\geq$1 $\log_2$ dilution lower MICs than BMD for 72.6% of our isolates, resulting in a shift toward susceptibility. The generally lower MTS MICs were more pronounced for isolates with low MICs. When the results were evaluated upon the performance criteria for susceptibility testing, MTS produced unacceptable results with marginally elevated VMEs with EUCAST breakpoints and increased mE rates with both FDA and EUCAST breakpoints.

Vitek2 is widely used in clinical laboratories. Our data highlight its important limitations in tigecycline susceptibility testing. It produced results with low EA and CA rates; ME and mE rates were severalfold higher than the acceptable performance with both FDA and EUCAST breakpoints and generally produced higher MICs, resulting in false resistance findings. These findings were more pronounced for CR bacteria (ME plus mE, >50%), against which, however, tigecycline therapy is more commonly

TABLE 3 Differences in $\log_2$ dilutions of MICs obtained by Vitek2, Etest, and MTS compared to BMD

Test method	No. (%) of isolates showing a MIC difference (in $\log_2$ dilutions) of:							
	>−3	−3	−2	−1	0	1	2	3
Vitek2				4 (1.7)	45 (18.7)	99 (41.1)	89 (36.5)	4 (1.7)
Etest		1 (0.4)	4 (1.7)	37 (15.4)	115 (47.7)	77 (32.0)	7 (2.9)	
MTS	5 (2.1)	6 (2.5)	43 (17.8)	121 (50.2)	56 (23.2)	10 (4.1)		

necessary. The only published study evaluating the performance of Vitek2 in the determination of the tigecycline susceptibility of KPC-producing *K. pneumoniae* strains reported a higher EA level and much lower mE rates than our results (15). These results could be explained by the lower MIC_{90}s for the isolates included in that study.

Major discrepancies were noted for CA and error rates between EUCAST and FDA data analyses, reflecting the high number of isolates for which the MICs were within ± 1 $\log_2$ dilution of the categorical breakpoints set by each organization.

Since tigecycline is commonly used against infections with CR pathogens, reliable susceptibility results are important for therapeutic decisions. Our study underlines the shortcomings of automated and manual susceptibility testing methods, which may falsely restrict the available treatment options or lead to inappropriate antimicrobial therapy. Clinical laboratories should be aware of the interpretive problems. Confirmation of susceptibility results by a reference method is therefore recommended, particularly when tigecycline administration is deemed necessary.

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