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Influence of Media and Testing Methodology on Susceptibility to Tigecycline of *Enterobacteriaceae* with Reported High Tigecycline MIC▽

M. Torrico,[1] N. González,[1] M. J. Giménez,[1] L. Alou,[1] D. Sevillano,[1] D. Navarro,[2] M. P. Díaz-Antolín,[3] N. Larrosa,[4] L. Aguilar,[1]* and N. Garcia-Escribano[5]

Microbiology Dpt., School of Medicine, Univ. Complutense, Avda. Complutense s/n, 28040 Madrid, Spain[1]; Microbiology Dpt., Hospital Clínico de Valencia, Avda. Blasco Ibañez 17, 46010 Valencia, Spain[2]; Microbiology Dpt., Hospital Son Llatzer, Ctra. Manacor, Km. 4, 07198 Palma de Mallorca, Spain[3]; Microbiology Dpt., Hospital Vall d'Hebron, Paseo Vall d'Hebron 119–129, 08035 Barcelona, Spain[4]; and Medical Department, Wyeth Farma S.A., Ctra. N-I, Km. 23 Desvío Algete Km. 1, San Sebastián de los Reyes, 28700 Madrid, Spain[5]

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The tigecycline susceptibility of six different *Enterobacteriaceae* strains with reported high tigecycline MICs was determined in quintuplicate by four methodologies using Mueller-Hinton agar and broth from six manufacturers. The MICs determined by Etest were a ≥1-fold dilution lower than those determined by broth microdilution and agar dilution, with the highest modal values given by agar dilution. The highest modal MICs were obtained using Oxoid medium, and the lowest inhibition zone values (disc diffusion) were obtained using Oxoid and bioMérieux media. The lowest MICs were obtained by Etest using Difco or Merck media.

As with any newly introduced antimicrobial agent, surveillances of susceptibility to tigecycline are critical to detect changes in its activity profile that depend on the testing methodology, on the medium used for susceptibility testing, and on the normal MIC distribution relative to the breakpoint value (12). Previous reports on susceptibility to tigecycline showed nearly 100% susceptibility in *Enterobacteriaceae* species, according to the breakpoints defined by the U.S. Food and Drug Administration (FDA) (12, 14). However, MIC values determined by Etest tended to be 1 double-dilution higher than those determined by broth microdilution (12). At least for *Acinetobacter* isolates, discrepancies were more pronounced in the group with higher MICs (3) and were clustered among the tigecycline-intermediate or -resistant strains (when MICs were determined by Etest) (4). In addition, MIC values two to eight times higher (and lower inhibition zone diameter values) have been reported for studies using media containing high manganese concentrations (9). The influence of the criteria used for interpretation (there are currently no defined CLSI breakpoints for tigecycline) was also reported in a previous study testing a limited number of strains, where susceptibility decreased from 100% and 95.7% (using FDA breakpoints) to 96.4% and 69.6% using the breakpoints defined by the British Society for Antimicrobial Chemotherapy (BSAC) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases for *Klebsiella pneumoniae* and *Enterobacter cloacae*, respectively (14).

The aim of this study was to assess the effect of combining different testing methodologies and test media, and of different breakpoints used for interpretation, on the susceptibility to tigecycline of six *Enterobacteriaceae* strains with reported tigecycline nonsusceptibility (>2 μg/ml) that were isolated in three different Spanish hospitals.

Two *K. pneumoniae* isolates with reported tigecycline MICs of 3 and 24 μg/ml, two *E. cloacae* isolates with reported MICs of 6 and 8 μg/ml, and two *Serratia marcescens* isolates with reported MICs of 4 and 8 μg/ml were studied. MICs were determined by Etest using commercially developed tigecycline Etest strips (AB Biodisk, Solna, Sweden) according to the instructions of the manufacturer (1) and by disc diffusion (using 15-μg tigecycline discs [Oxoid Ltd., Basingstoke, Hampshire, United Kingdom]), broth microdilution, and agar dilution following CLSI recommendations (5, 6). All tests were performed using Mueller-Hinton broth (for broth microdilution) or agar (for Etest, disc diffusion, and agar dilution) from Difco (BD Diagnostic Systems, Sparks, MD) (batch no. 275730 for broth and batch no. 218747 for agar), Merck (Darmstadt, Germany) (batch no. VL009693 833 for broth and batch no. FN1092635 929 for agar), Remel (Thermo Fisher Scientific, Lenexa, KS) (batch no. 774389 for broth and batch no. 655291 for agar), BBL (BD Diagnostic Systems, Sparks, MD) (batch no. 9044411 for broth and batch no. 9174125 for agar), and Oxoid (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) (batch no. 724245 for broth and batch no. 784397 for agar). In addition, prepared Mueller-Hinton plates from bioMérieux (Marcy l'Etoile, France) (batch no. 11870 and batch no. 11871) were also used for Etest and disc diffusion tests, respectively. To avoid the reported effect of medium age on the determination of *in vitro* activity of tigecycline by broth microdilution (11), all prepared media were used within 24 h.

Each testing methodology with each medium was performed in quintuplicate for each strain, and modal MIC values and range, or means ± standard deviations (SD) (in millimeters) of inhibition zone diameters, were calculated. *Escherichia coli* ATCC 25922 was used as the control strain. Breakpoints considered for interpretation were those defined by the FDA (FDA-approved drug products [http://www.accessdata.fda.gov/Scripts

* Corresponding author. Mailing address: Microbiology Dept., School of Medicine, Universidad Complutense, Avda. Complutense s/n, 28040 Madrid, Spain. Phone: 34-91-3941505. Fax: 34-91-3941511. E-mail: laguilar@med.ucm.es.

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TABLE 1. Tigecycline MICs determined by different methods, inhibition zone diameter determinations (disc diffusion), and susceptibility categorization using different media for *E. coli* ATCC 25922 (five replicates for each method with each medium)[a]

Medium	Etest MIC (μg/ml) [modal value (range)]	Disc diffusion zone diam (mm; mean ± SD)[b]	Broth dilution MIC (μg/ml) [modal value (range)]	Agar dilution MIC [modal value (range)]
Difco	0.09 (0.09–0.12)	25.9 ± 0.3	0.12 (0.12–0.25)	0.25 (0.25–0.25)
Merck	0.09 (0.09–0.09)	26.8 ± 0.2	0.25 (0.25–0.5)	0.12 (0.12–0.12)
Remel	0.19 (0.19–0.19)	24.8 ± 0.3	0.25 (0.25–0.5)	0.5 (0.25–0.5)
BBL	0.19 (0.19–0.25)	24.0 ± 0.2	0.12 (0.12–0.25)	0.25 (0.25–0.25)
Oxoid	0.19 (0.19–0.19)	22.3 ± 0.1*	0.25 (0.25–0.5)	0.5 (0.25–0.5)
bioMérieux	0.25 (0.25–0.38)	21.9 ± 0.4*		

[a] BSAC/EUCAST results are represented as follows: no symbol, susceptible; asterisk, intermediate. The BSAC/EUCAST MIC susceptibility and resistance breakpoints were ≤1 μg and ml≥4 μg/ml; the BSAC/EUCAST disc diffusion zone diameter susceptibility and resistance breakpoints were ≥24 mm and ≤19 mm.
[b] Mean values were rounded up or down to the nearest digit for susceptibility interpretations.

/cder/DrugsatFDA/; last access on 8 January 2010]), the British Society for Antimicrobial Chemotherapy (BSAC [http://www.bsac.org.uk/db_documents/Version_9.1_March_2010_final.pdf; last access on 8 January 2010]), and EUCAST (European Society of Clinical Microbiology and Infectious Diseases [http://www.srga.org/eucastwt/MICTAB/MICtetracyclines.htm; last access on 23 April 2010]).

Table 1 shows results for *E. coli* ATCC 25922 (control strain). Good reproducibility for each testing methodology with each media was obtained, since MIC values were always within 1 dilution, and standard deviations for inhibition zone diameters were <0.5 mm in the quintuplicate testing. While quality control inhibition zones were always within acceptable limits (20 to 27 mm in diameter) (7) regardless of the medium used, mean quality control MIC values determined by agar dilution using Remel or Oxoid media were not within acceptable limits (0.03 to 0.25 μg/ml) (7). Mean MIC values determined by agar dilution were higher than those determined by Etest. MIC values were always within the susceptibility range regardless of the medium used. The lowest mean inhibition zone diameter values were obtained using media from Oxoid and bioMérieux, leading to classification of the control strain *E. coli* ATCC 25922 as intermediate-resistant according to the BSAC/EUCAST breakpoints but as susceptible according to the FDA breakpoints.

Table 2, Table 3, and Table 4 show the results for the six study strains. In general, good reproducibility (values within ±1 dilution) was found for each testing methodology with each medium in the 5-fold determinations performed. Differences arose in comparisons of the MIC values determined by the different combinations of methods and media tested, leading to different susceptibility categorizations of the strains.

Table 2 shows results for the *K. pneumoniae* strains. Modal MIC values ranged from 2 to 8 μg/ml for *K. pneumoniae* strain 1 and from 6 to 16 μg/ml for *K. pneumoniae* strain 2, depending on the testing methodology or the medium used. All modal MIC values were within the nonsusceptibility range except for the modal MIC for strain 1 determined by Etest using the Merck medium according to the FDA breakpoints (resulting in classification of the strain as susceptible). Table 3 shows results for the *E. cloacae* strains, with modal MIC values ranging from 2 to 8 μg/ml for both strains, depending on the testing methodology or the medium used. Modal values were within the nonsusceptibility range except for the modal MIC values for strain 1 determined by Etest using Difco or Merck media or by broth microdilution using Difco and Remel media and using the FDA breakpoints for categorization. Using the same breakpoints, the same result occurred with modal MIC values for strain 2 when MICs were determined by Etest using the Difco medium. Table 4 shows results for *S. marcescens*, with modal values ranging from 0.75 to 8 μg/ml for both strains, depending on the testing methodology or the medium used. In general, using FDA breakpoints, modal MIC values were within the nonsusceptibility range only when MICs were de-

TABLE 2. Tigecycline MICs determined by different methods, inhibition zone diameter determinations (disc diffusion), and susceptibility categorization using different media for *Klebsiella pneumoniae* strains (five replicates for each method with each medium)[a]

Medium	*K. pneumoniae* strain 1				*K. pneumoniae* strain 2			
	Etest MIC (μg/ml) [modal value (range)]	Disc diffusion zone diam (mm; mean ± SD)[b]	Broth dilution MIC (μg/ml) [modal value (range)]	Agar dilution MIC (μg/ml) [modal value (range)]	Etest MIC (μg/ml) [modal value (range)]	Disc diffusion zone diam (mm; mean ± SD)[b]	Broth dilution MIC (μg/ml) [modal value (range)]	Agar dilution MIC (μg/ml) [modal value (range)]
Difco	3 (2–3)*I	16.6 ± 1.1I	4 (2–8)I	4 (4–4)I	6 (4–6)I	14.8 ± 0.2I	8 (4–8)R	16 (8–16)R
Merck	2 (1.5–3)*S	16.7 ± 1.1I	4 (4–8)I	4 (4–4)I	6 (4–6)I	14.1 ± 0.1R	16 (16–16)R	16 (16–16)R
Remel	3 (3–3)*I	15.7 ± 0.5I	4 (4–8)I	8 (4–8)R	8 (8–8)R	13.6 ± 0.3R	8 (8–16)R	16 (16–16)R
BBL	3 (3–3)*I	15.5 ± 0.5I	4 (4–8)I	8 (4–8)R	8 (6–12)R	13.1 ± 0.2R	8 (8–16)R	16 (16–16)R
Oxoid	4 (4–6)I	14.1 ± 0.6R	8 (8–16)R	8 (8–8)R	8 (8–8)R	12.8 ± 0.3R	8 (8–32)R	16 (16–16)R
bioMérieux	3 (3–4)I	13.4 ± 0.6R			12 (12–12)R	12.6 ± 0.3R		

[a] For susceptibility determinations according to the FDA guidelines, "S" indicates susceptibility, "I" indicates intermediate susceptibility, and "R" indicates resistance. The FDA MIC susceptibility and resistance breakpoints were ≤2 μg/ml and ≥8 μg/ml, respectively; the FDA disc diffusion zone diameter susceptibility and resistance breakpoints were ≥19 mm and ≤14 mm, respectively. For BSAC/EUCAST susceptibility determinations, the absence of a symbol indicates susceptibility, an asterisk indicates intermediate susceptibility, and underlining indicates resistance. The BSAC/EUCAST MIC susceptibility and resistance breakpoints were ≤1 μg/ml and ≥4 μg/ml, respectively; the BSAC/EUCAST disc diffusion zone diameter susceptibility and resistance breakpoints were ≥24 mm and ≤19 mm, respectively.
[b] Mean values were rounded up or down to the nearest digit for susceptibility interpretations.

TABLE 3. Tigecycline MICs determined by different methods, inhibition zone diameter determinations (disc diffusion), and susceptibility categorization using different media for *Enterobacter cloacae* strains (five replicates for each method with each medium)[a]

Medium	*E. cloacae* 1				*E. cloacae* 2			
	Etest MIC (μg/ml) [modal value (range)]	Disc diffusion zone diam (mm; mean ± SD)[b]	Broth dilution MIC (μg/ml) [modal value (range)]	Agar dilution MIC (μg/ml) [modal value (range)]	Etest MIC (μg/ml) [modal value (range)]	Disc diffusion zone diam (mm; mean ± SD)[b]	Broth dilution MIC (μg/ml) [modal value (range)]	Agar dilution MIC (μg/ml) [modal value (range)]
Difco	2 (2–2)*S	16.2 ± 0.8I	2 (2–4)*S	4 (4–8)I	2 (2–3)*S	16.7 ± 0.6I	4 (4–8)I	8 (4–8)R
Merck	2 (2–2)*S	16.5 ± 0.3I	4 (4–8)I	4 (4–4)I	3 (1.5–3)*I	16.4 ± 0.1I	8 (4–8)R	4 (4–4)I
Remel	3 (3–3)*I	15.1 ± 0.6I	2 (2–8)*S	4 (4–8)I	3 (3–3)*I	16.4 ± 0.3I	4 (4–8)I	8 (4–8)R
BBL	3 (3–3)*I	15.9 ± 0.2I	4 (2–4)I	8 (4–8)R	4 (3–4)I	15.3 ± 0.4I	8 (4–8)R	8 (4–8)R
Oxoid	4 (4–6)I	14.7 ± 0.6I	4 (4–8)I	8 (4–8)R	3 (3–4)*I	14.8 ± 0.4I	8 (4–16)R	8 (4–8)R
bioMérieux	4 (3–4)I	15.1 ± 0.5I			4 (4–4)I	14.5 ± 0.3I		

[a] For susceptibility determinations according to the FDA guidelines, "S" indicates susceptibility, "I" indicates intermediate susceptibility, and "R" indicates resistance. The FDA MIC susceptibility and resistance breakpoints were ≤2 μg/ml and ≥8 μg/ml, respectively; the FDA disc diffusion zone diameter susceptibility and resistance breakpoints were ≥19 mm and ≤14 mm, respectively. For BSAC/EUCAST susceptibility determinations, the absence of a symbol indicates susceptibility, an asterisk indicates intermediate susceptibility, and underlining indicates resistance. The BSAC/EUCAST MIC susceptibility and resistance breakpoints were ≤1 μg/ml and ≥4 μg/ml, respectively; the BSAC/EUCAST disc diffusion zone diameter susceptibility and resistance breakpoints were ≥24 mm and ≤19 mm, respectively.
[b] Mean values were rounded up or down to the nearest digit for susceptibility interpretations.

termined by agar dilution for both strains or by broth microdilution using the Merck or Oxoid media. When BSAC and EUCAST breakpoints are considered, modal MIC values were within the nonsusceptibility range in all cases except for the MICs determined by Etest using the Merck medium for strain 1 or Difco, Merck, Remel, and BBL media for strain 2.

Overall, for all strains, MICs determined by broth microdilution and agar dilution were at least 1-fold higher than those determined by Etest, with the highest MIC values obtained by agar dilution. With respect to media, the highest modal MIC values were obtained using the media from Oxoid, and the lowest inhibition zone diameters were obtained using media from Oxoid and bioMérieux. Analysis of combinations of testing methodologies and media showed that the lowest MICs were obtained by Etest using the Difco or Merck media.

A previous study testing tigecycline-susceptible *K. pneumoniae* reported higher MICs with Merck versus Difco or Oxoid media, attributing this effect to the higher manganese content in Merck's medium (630 ppm versus 2.5 ppm) (9), while other authors testing *Acinetobacter* isolates reported lower susceptibility rates with Oxoid versus Becton-Dickinson media, also attributing this effect to the fact that the manganese content in the Oxoid medium was three times greater (13). In the current study, the lowest inhibition zone diameter values in the disc diffusion test were obtained using media from Oxoid and bioMérieux. The content in other ions could have some influence. Media from Merck and Difco have lower Mg content than Oxoid media (around 195 versus 409 ppm) (9), and it has been reported that magnesium and calcium interfere with the activity of tetracyclines, aminoglycosides, and colistin against *Pseudomonas aeruginosa* and other Gram-negative bacteria (8, 15).

In a previous study employing susceptibility testing of *Acinetobacter* spp. to compare MIC values determined by Etest (using media from bioMérieux) versus broth microdilution (using media from Remel), Etest-intermediate or -resistant strains were susceptible when tested by broth microdilution (using BSAC and EUCAST breakpoints) (4). However, since higher Etest MIC values have been reported in studies of *Acinetobacter* spp. with Mueller-Hinton agar from bioMérieux (2), differences could be attributed to different methodologies or different media. In the current study testing *Enterobacteriaceae* strains with reported high tigecycline MICs, MICs determined by Etest using media from bioMérieux were in most

TABLE 4. Tigecycline MICs determined by different methods, inhibition zone diameter determinations (disc diffusion), and susceptibility categorization using different media for *Serratia marcescens* strains (five replicates for each method with each medium)[a]

Medium	*S. marcescens* 1				*S. marcescens* 2			
	Etest MIC (μg/ml) [modal value (range)]	Disc diffusion zone diam (mm; mean ± SD)[b]	Broth dilution MIC (μg/ml) [modal value (range)]	Agar dilution MIC (μg/ml) [modal value (range)]	Etest MIC (μg/ml) [modal value (range)]	Disc diffusion zone diam (mm; mean ± SD)[b]	Broth dilution MIC (μg/ml) [modal value (range)]	Agar dilution MIC (μg/ml) [modal value (range)]
Difco	1.5 (1.5–1.5)*S	21.8 ± 0.6*S	2 (1–2)*S	4 (2–4)I	1 (1–1.5)S	21.9 ± 0.7*S	2 (1–4)*S	4 (2–8)I
Merck	0.75 (0.75–0.75)S	22.6 ± 0.9*S	4 (4–8)I	2 (2–4)*S	0.75 (0.75–1)S	23.1 ± 0.5*S	8 (4–8)R	4 (4–4)I
Remel	2 (1.5–2)*S	19.9 ± 0.2*S	1 (1–4)S	4 (4–8)I	1 (1.5–1.5)S	21.6 ± 0.7*S	2 (2–8)*S	8 (4–8)R
BBL	2 (1.5–2)*S	19.8 ± 0.4*S	2 (2–4)*S	4 (4–8)I	1 (0.5–1)S	21.3 ± 0.4*S	2 (2–4)*S	8 (4–8)R
Oxoid	2 (2–3)*S	18.4 ± 0.4I	8 (4–8)R	8 (4–8)R	2 (1.5–2)*S	19.1 ± 0.2*S	4 (4–8)I	8 (4–8)R
bioMérieux	3 (3–3)I	17.7 ± 0.4I			2 (1.5–2)*S	19.2 ± 0.2*S		

[a] For susceptibility determinations according to the FDA guidelines, "S" indicates susceptibility, "I" indicates intermediate susceptibility, and "R" indicates resistance. The FDA MIC susceptibility and resistance breakpoints were ≤2 μg/ml and ≥8 μg/ml, respectively; the FDA disc diffusion zone diameter susceptibility and resistance breakpoints were ≥19 mm and ≤14 mm, respectively. For BSAC/EUCAST susceptibility determinations, the absence of a symbol indicates susceptibility, an asterisk indicates intermediate susceptibility, and underlining indicates resistance. The BSAC/EUCAST MIC susceptibility and resistance breakpoints were ≤1 μg/ml and ≥4 μg/ml, respectively; the BSAC/EUCAST disc diffusion zone diameter susceptibility and resistance breakpoints were ≥24 mm and ≤19 mm, respectively.
[b] Mean values were rounded up or down to the nearest digit for susceptibility interpretations.

cases higher than those determined by Etest using other media, while MICs determined by broth microdilution were in general 1 dilution higher than those determined by Etest (when using the same media). This discrepancy greatly increased when determining MICs by agar dilution.

High rates of susceptibility to tigecycline in *Enterobacteriaceae* strains have been reported in the literature (10, 12), with a low number of strains showing high tigecycline MIC values. The results of this study, although limited by the low number of strains tested (because of the rarity of high MICs currently seen in practice), show that caution should be taken in interpreting results from MIC testing of *Enterobacteriaceae* strains when high MICs are obtained, since susceptibility categorization depends not only on the breakpoints used but also on the susceptibility test and media used.

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