In Vitro Activity of Tigecycline against Gram-Positive and Gram-Negative Pathogens as Evaluated by Broth Microdilution and Etest

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The current surveillance establishes the activity profile of tigecycline against recent clinical U.S. isolates of target pathogens. Findings from a distributed surveillance that utilized Etest yielded a tigecycline activity profile that varied from that observed in a separate centralized broth microdilution (BMD) surveillance (D. C. Draghi et al., Poster D-0701, 46th Intersci. Conf. Antimicrob. Agents Chemother., San Francisco, CA). Differences were noted among Acinetobacter spp. and Serratia marcescens and, to a lesser extent, with Streptococcus pyogenes. To address whether these differences were due to discordance in testing methodology or to variations among the analyzed populations, isolates from the current surveillance were concurrently tested by BMD and Etest. In all, 1,800 Staphylococcus aureus, 259 S. pyogenes, 226 Streptococcus pneumoniae, 93 Enterococcus faecalis, 1,356 Enterobacteriaceae, and 227 Acinetobacter baumannii strains were evaluated. Tigecycline had potent activity by BMD, with >99.6% susceptibility (%S) observed for all pathogens with interpretive criteria, excluding Enterobacter cloacae (98.3% S) and E. faecalis (86.0% S), and MIC\textsubscript{90} ranged from 0.03 μg/ml (S. pyogenes/S. pneumoniae) to 1 μg/ml (Enterobacteriaceae/A. baumannii). Similar profiles were observed by Etest, with the exception of A. baumannii, although for most evaluated pathogens Etest MICs tended one doubling-dilution higher than BMD MICs. Major or very major errors were infrequent, and a high degree of essential agreement was observed, excluding A. baumannii, S. marcescens, and S. pneumoniae, for which ≥4-fold differences in MICs were observed for 29, 27.1, and 34% of the isolates, respectively. Further analysis regarding the suitability of the tigecycline Etest for testing S. marcescens, Acinetobacter spp., and S. pneumoniae is warranted.

Tigecycline is a novel semisynthetic glycylcycline with broad-spectrum activity against a variety of clinically relevant organisms including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae*, beta-hemolytic streptococci, enterococci (vancomycin-susceptible isolates), *Enterobacteriaceae* (including extended-spectrum beta-lactamase isolates), *Acinetobacter* spp., and anaerobes (*Bacteroides* spp., peptostreptococci, and *Clostridium perfringens*) (3, 14, 17). Tigecycline was approved in 2005 by the U.S. Food and Drug Administration (FDA) and in 2006 by the European Medicines Agency for the treatment of complicated skin and skin structure infections and complicated intra-abdominal infections and is currently being evaluated for the treatment of hospital- and community-acquired pneumonia.

As a new agent, surveillance of the activity of tigecycline against its target pathogens has been widely reported (2, 6, 9, 10, 18) and is critical for both its ongoing clinical development and for the detection of any changes in susceptibility patterns. Surveillance programs can be accomplished in a variety of fashions (distributed, local network reporting, and centralized) and can use a variety of testing methodologies (broth microdilution, Etest, disk diffusion, and automated systems). As the reference standard, broth microdilution testing serves as the method of comparison for the development and evaluation of alternative susceptibility testing methodologies (12, 15).

Recently, an Etest has been developed for the susceptibility testing of tigecycline (1). In a distributed surveillance study where the susceptibility of tigecycline against target pathogens was determined locally by Etest (32 participating institutions), the activity profile observed for tigecycline varied from that observed in a recent centralized surveillance which utilized BMD (7). In brief, the MIC\textsubscript{90} for *Acinetobacter* spp. and *Serratia marcescens* was fourfold higher by distributed Etest relative to that observed in centralized BMD testing. A difference in profile between the two methods was less apparent for *Streptococcus pyogenes*, although Etest MICs tended to be one doubling dilution higher in the distributed Etest surveillance compared to those in the centralized BMD surveillance. Tigecycline activity profiles against the remaining target organisms evaluated were similar for both surveillances. To better understand whether the difference in activity profiles between the two surveillances mentioned above could be attributed to variations in testing methodology or were due to variable activity against two distinct populations of isolates, the current surveillance initiative profiles tigecycline activity against recent clinical isolates of its target pathogens via the concurrent testing of these isolates in a central laboratory by both Etest and BMD.

**MATERIALS AND METHODS**

**Bacteria.** Clinical isolates from 97 participating sites geographically distributed across the nine U.S. Bureau of Census regions were collected from 2006 to 2007 and were shipped to Eurofins Medinet, Inc. (Herndon, VA), for centralized susceptibility testing by both BMD and Etest. Isolates were selected to consist of a variety of clinically relevant resistance phenotypes (e.g., penicillin-resistant pneumococci and MRSA). *S. pneumoniae* interpretive criteria for penicillin according to the Clinical and Laboratory Standards Institute (CLSI; ≤0.06 μg/ml =
TABLE 1. Tigecycline surveillance by centralized BMD

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>Centralized surveillance result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC (µg/ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1,800</td>
<td>0.03–1</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>259</td>
<td>0.015–0.06</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>226</td>
<td>≤0.008–0.06</td>
</tr>
<tr>
<td>E. faecalisb</td>
<td>93</td>
<td>0.03–0.5</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>1,356</td>
<td>0.06–4</td>
</tr>
<tr>
<td>E. coli</td>
<td>452</td>
<td>0.06–2</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>450</td>
<td>0.12–4</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>178</td>
<td>0.06–2</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>180</td>
<td>0.12–4</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>96</td>
<td>0.25–2</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>227</td>
<td>0.03–2</td>
</tr>
</tbody>
</table>

* A dash (–) indicates that breakpoints are currently unavailable for interpretation as susceptible (S), intermediate (I), and/or resistant (R).

b An FDA susceptible breakpoint of ≤0.25 µg/ml for E. faecalis (vancomycin susceptible) was used to interpret tigecycline results for all E. faecalis.

sustainable; 0.12 to 1 µg/ml = intermediate; ≥2 µg/ml = resistant) were utilized for selection. In all, 4,051 isolates were tested consisting of 1,800 S. aureus (70% MRSA), 259 S. pyogenes, 226 S. pneumoniae (10% penicillin resistant), 93 Enterococcus faecalis (98.9% vancomycin susceptible), 1,356 Enterobacteriaceae (452 Escherichia coli, 450 Klebsiella pneumoniae, 178 Citrobacter spp., 180 Enterobacter spp., and 96 S. marcescens), and 227 Acinetobacter baumannii. Upon receipt, the identification of each isolate was confirmed by standard microbiological protocols (11, 13), and each isolate was subcultured to purity on 5% sheep blood agar prior to preparing the inoculum for susceptibility testing.

Susceptibility testing and interpretation. Antimicrobial susceptibility testing was performed centrally at Eurofins Medinet, Inc., by broth microdilution as directed by CLSI document M7-A7 (4) using commercial frozen panels (TREK Diagnostics, Cleveland, OH) and Mueller-Hinton broth (Remel). Susceptibility testing by Etest was also performed concurrently at Eurofins Medinet, Inc., using commercially developed tigecycline Etest strips (AB Biodisk, Solna, Sweden) according to the manufacturer’s instructions (1).

After appropriate incubation, MICs were read at 100% inhibition of growth, and categorical interpretations of susceptible, intermediate, or resistant for tigecycline were applied using the FDA interpretive breakpoints detailed in the Tigacil package insert on the FDA website (http://www.fda.gov/iceci/foi/label/2007/021826s010lbl.pdf). S. aureus, susceptible ≤ 0.5 µg/ml; streptococci (non-pneumococci) and E. faecalis (vancomycin-susceptible E. faecalis breakpoints were applied for all enterococci), susceptible ≤ 0.25 µg/ml; and Enterobacteriaceae, susceptible ≤ 2 µg/ml, intermediate = 4 µg/ml, resistant ≥ 8 µg/ml. FDA breakpoints were applied due to the lack of established CLSI breakpoints for tigecycline at this time. Throughout the study, S. pneumoniae ATCC 49619, S. aureus ATCC 29213, E. faecalis ATCC 29212, E. coli ATCC 25922, and P. aeruginosa ATCC 27853 were used for quality control as appropriate in accordance with CLSI document M100-S17 (5).

RESULTS

Tigecycline surveillance by centralized BMD testing. The current level of activity of tigecycline against the tested gram-positive and gram-negative clinical isolates by centralized BMD surveillance is depicted in Table 1. The tested isolates were >99.6% susceptible to tigecycline with the exception of Enterobacter spp. (98.3% susceptible) and E. faecalis (86% susceptible). Among the tested gram-positive isolates, tigecycline was most active as reflected by MIC50/MIC90 values against S. pyogenes (0.03/0.03 µg/ml) and S. pneumoniae (0.015/0.03 µg/ml), followed by S. aureus (0.12/0.25 µg/ml) and E. faecalis (0.12/0.5 µg/ml). The activity of tigecycline as reflected by MIC50/MIC90 was unaltered against MRSA (0.12/0.5 µg/ml), macrolide-resistant S. pyogenes (0.03/0.03 µg/ml), and penicillin-resistant pneumococci (0.015/0.03 µg/ml) relative to susceptible isolates (data not shown). A total of 7 of the 1,800 S. aureus (0.4%); 5 MRSA and 2 MSSA) were tigecycline nonsusceptible, with MICs of 1 µg/ml.

Against the tested gram-negative isolates, tigecycline had consistent activity based on the MIC50 and MIC90. Against species of Enterobacteriaceae and A. baumannii, an MIC50 between 0.25 and 0.5 µg/ml and an MIC90 between 0.5 and 1 µg/ml was observed. No Enterobacteriaceae isolates were resistant to tigecycline although 1.7 and 0.2% of Enterobacter spp. and K. pneumoniae, respectively, were intermediate (had tigecycline MICs of 4 µg/ml). Tigecycline MICs did not exceed 2 µg/ml against the tested isolates of A. baumannii.

Concurrent testing of isolates by centralized BMD and centralized Etest. To better determine whether the discrepancies in tigecycline activity noted between a prior distributed surveillance conducted by Etest and other centralized BMD surveillances were due to any discordance between the two testing methods or rather to differing susceptibility of the population of isolates tested in both studies, isolates in the present study were tested by both BMD and Etest.

The activity of tigecycline as evaluated by Etest is presented in Table 2. The MIC50 as determined by Etest of all tested isolates was either identical or within one doubling dilution of the MIC90 of isolates tested by BMD (Table 1), with the exception of A. baumannii (MIC50: Etest = 4 µg/ml, BMD = 1 µg/ml). The MIC50s were one doubling dilution higher among the tested streptococci, S. marcescens and K. pneumoniae, by Etest relative to BMD, while the MIC90 of S. aureus was one doubling dilution lower by Etest than by BMD. In addition, the MIC50 of S. marcescens was fourfold higher by Etest than by BMD (MIC50: Etest = 2 µg/ml, BMD = 0.5 µg/ml). By MIC distribution, the discordance between Etest and BMD was apparent for A. baumannii (Fig. 1A), and Etest MICs trended one doubling dilution higher than BMD MICs against S. pyogenes (Fig. 1B), S. pneumoniae (Fig. 1C), and S. marcescens (Fig. 1D). For A. baumannii, S. pyogenes, and S. pneumoniae, there was a substantial portion of isolates with Etest MICs that were outside the normal distribution observed with BMD MICs (Fig. 1A to C). The differences in testing methodology were not attributable to interpretation errors since tigecycline tested by Etest yielded a clear ellipse and by broth microdilution reading was based on 100% inhibition of
growth. Notable differences in Etest and BMD MIC distributions among the other evaluated species (S. aureus, E. faecalis, and Enterobacteriaceae overall) were not observed (data not shown).

**Evaluation of Etest performance among target pathogens.**

The essential agreement between Etest and BMD and the Etest error rate observed in the present study is depicted in Table 3. Essential agreement was reported as the percentage of isolates for which the Etest MIC was the same or one doubling dilution apart from the BMD MIC. As the variations in MIC50/MIC90 noted above suggest, low essential agreement between the Etest and BMD was observed among the tested A. baumannii (65.6%) and S. marcescens (72.9%) strains. Somewhat unexpectedly, only 69.6% essential agreement was observed for S. pneumoniae and was largely attributed to Etest MICs that registered fourfold higher than their respective BMD MIC.

Etest MICs were generally two- to fourfold higher than BMD MICs among the tested pathogens, with the exception of S. aureus, where Etest MICs were two- to fourfold lower than BMD MICs for 44% of the tested isolates, and the tested streptococci, where Etest MICs among 4 to 5% of the isolates were eightfold greater than their corresponding BMD MICs. A linear correlation ($R$ at or near 1) between the two methods

![Figure 1](http://jcm.asm.org/)

**FIG. 1.** MIC distribution of tigecycline against A. baumannii (A), S. pyogenes (B), S. pneumoniae (C), and S. marcescens (D).
was noted mainly among *Enterobacteriaceae* (excluding *S. marcescens*), *E. faecalis* and, surprisingly, *A. baumannii*. Less linearity was observed among the tested *S. aureus* (*R* = 0.478) and in particular among the tested streptococci (*R* = 0.175 for *S. pyogenes* and *R* = 0.153 for *S. pneumoniae*). Importantly, few significant errors in interpretation were evident, although error rates could not be determined for *A. baumannii* or *S. pneumoniae* due to the absence of interpretive criteria for these organisms. A 5% major error rate (false nonsusceptible by Etest) was noted for the testing of *S. pyogenes*, and a 6.5% very major error rate (false susceptible by Etest) was noted among the tested *E. faecalis*. It is important to note that 100% of the *E. faecalis* isolates that resulted in a very major error were one doubling dilution above the susceptibility breakpoint by BMD and one doubling dilution below the susceptibility breakpoint by Etest.

### DISCUSSION

As an agent for the treatment of complicated intra-abdominal infections and skin structure infections, tigecycline is used primarily in hospitals where emerging resistance is often a concern. As a new agent designated for use in a nosocomial environment, the constant monitoring of tigecycline activity against pathogens for which it is indicated is of heightened importance. Surveillance of tigecycline against target pathogens has been addressed by multiple ongoing centralized surveillance initiatives (2, 10, 16, 18), where geographically distributed isolates are tested by BMD according to CLSI criteria (4, 5).

As commercial methods (e.g., disk diffusion, Etest, etc.) are developed and become more available as alternatives for the susceptibility testing of new agents, it is important to know both the validity of these methods and whether or not there is any impact of data generated from widespread testing by these methods on the reported activity profile of the drug. Both centralized surveillance testing using standardized conventional BMD, and routine testing on local levels using commercial methods provide important insights on the activity of tigecycline. Both approaches to monitoring activity are key information resources, and there is a need to understand the extent to which the data provided by each approach are concordant and to identify the nature of any discordance that may be encountered.

There was apparent discordance in the tigecycline activity profile obtained from a recent distributed Etest surveillance relative to a centralized BMD surveillance (7); however, it was not clear whether this discordance was due to variation in testing methodology between the two studies or variation in the analyzed population of isolates. The current surveillance study utilized BMD and Etest both to establish the current activity profile of tigecycline against U.S. isolates of target pathogens and to allow for a direct assessment of the potential variability between the two testing methods.

Overall, tigecycline exhibited potent in vitro activity by BMD against the indication-relevant pathogens, and the overall pattern of activity by MIC₉₀ was similar to previously published tigecycline surveillance studies (2, 9, 10, 16, 18), with potent activity against resistant gram-positive isolates and activity against *Enterobacteriaceae* and *Acinetobacter* spp. Interestingly, the MIC₉₀ against *S. pneumoniae* (0.03 μg/ml) by BMD was lower than that reported in other U.S.-based surveillances (2, 10), while the MIC₉₀ against *E. faecalis* (0.5 μg/ml by BMD) was slightly higher (10).

Similar activity profiles should be observed when testing the same isolates by different methods, as was the case for most of the pathogens evaluated by Etest and BMD based on MIC₉₀/MIC₉₀ and percent susceptibility. However, there were some notable exceptions. The most apparent difference between the two testing methodologies was noted to occur among the tested *A. baumannii*, where a fourfold increase in MIC₉₀ was observed by Etest relative to BMD. By MIC distribution, a large proportion of isolates (23%) had Etest MICs one to two
doubling dilutions higher than the upper limit observed by BMD MIC. This discordance was reflected in a low essential agreement between the two methods. Although no interpretive criteria are yet available for the assessment of tigecycline activity against *Acinetobacter* spp., based on the current data there is potential for a large degree of major errors in tigecycline susceptibility interpretation by Etest when this species is tested due to the apparent discordance with BMD results. Although it is difficult to pinpoint what factors may be affecting Etest performance against *Acinetobacter* spp., a recent study has noted that increasing concentrations of manganese in Mueller-Hinton agar resulted in increased tigecycline MICs against *A. baumannii* by Etest (8). Thus, variation in manganese content among separate lots of media from the same manufacturer or lots of media between manufacturers can have a significant impact on the susceptibility testing of *Acinetobacter* spp. by tigecycline Etest. This impact would be of particular significance in local reporting and distributed surveillance studies in which the tigecycline Etest is utilized.

Although less apparent than the differences observed between Etest and BMD for *A. baumannii*, Etest MICs tended to be somewhat higher than BMD MICs overall, with the exception of *S. aureus*, for which a number of isolates had lower MICs by Etest. This difference translated to less-discernible differences between the two methods by MIC$_{50}$s (which were within one doubling dilution) and percent susceptibility, values which were similar for both methods. However, for *S. marcescens*, *S. pyogenes*, and *S. pneumoniae*, this slight difference was more apparent by overall MIC distribution. This disparity correlated to a low rate of essential agreement between the two methods for *S. marcescens* and *S. pneumoniae*, a 5% major error rate for *S. pyogenes*, and a low degree of linearity between the two methods for these species. This is in direct contrast to an Etest validation study where a high degree of essential agreement was noted between both methods for both *S. pneumoniae* and nonpneumococcal streptococci (1). The cause for this disparity between the two studies is unknown and warrants further investigation, but important factors could include strain variability and interlaboratory variability (interpretation, materials, etc.).

In summary, tigecycline had potent activity against the target pathogens analyzed, including relevant resistant isolates of gram-positive cocci. Slightly different tigecycline activity profiles were noted between isolates tested by Etest and BMD primarily among *A. baumannii* and to a lesser extent among *S. marcescens*, *S. pneumoniae*, and *S. pyogenes*. Although a less dramatic difference was observed for other species, it is important to note that even a one-doubling-dilution difference can affect the activity profile, depending on where the normal distribution is relative to the breakpoint. Nonetheless, the discordance observed by direct comparison of testing methodology in the present study identified testing issues for Etest with the same target pathogens in which varied activity profiles were observed for tigecycline by distributed Etest in a prior study (7). Thus, it seems likely that variations in testing methodology, particularly for *Acinetobacter* spp., played a significant role in the discrepant activity profiles between the two studies (distributed Etest versus centralized BMD), although the contribution of differing susceptibilities among the populations of isolates to the observed variation cannot be measured. The issue regarding Etest testing of *Acinetobacter* spp. has already received some attention by other investigators (8), and their results, combined with the noted discordance with BMD results observed for *Acinetobacter* spp. in the present study and others (7), raise concerns regarding the suitability of using Etest for determining the susceptibility of *Acinetobacter* spp. to tigecycline. The present study also noted low essential agreement for the Etest against *S. pneumoniae* and *S. marcescens*. A portion (ca. 5%) of *S. pneumoniae* and *S. pyogenes* had Etest MICs at least eightfold higher than their corresponding BMD MICs. Since the tigecycline Etest has previously been validated against streptococci, further examination into why discordance was observed in the present study among these species should be considered. In addition, further evaluation of the Etest against *Acinetobacter* spp., streptococci, and *S. marcescens* in particular should proceed if the Etest is to be considered a viable alternative for susceptibility testing against these organisms. Until data from further studies regarding the suitability of the tigecycline Etest against *Acinetobacter* spp., streptococci, and *S. marcescens* become available, caution is warranted in utilizing tigecycline Etest data for these organisms, in particular with respect to *Acinetobacter* spp., where a variable manganese concentration in media has been shown to affect results (8).

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