Agreement Assessment of Tigecycline Susceptibilities Determined by the Disk Diffusion and Broth Microdilution Methods among Commonly Encountered Resistant Bacterial Isolates: Results from the Tigecycline In Vitro Surveillance in Taiwan (TIST) Study, 2008 to 2010


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The Tigecycline In Vitro Surveillance in Taiwan (TIST) study, initiated in 2006, is a nationwide surveillance program designed to longitudinally monitor the in vitro activity of tigecycline against commonly encountered drug-resistant bacteria. This study compared the in vitro activity of tigecycline against 3,014 isolates of clinically important drug-resistant bacteria using the standard broth microdilution and disk diffusion methods. Species studied included methicillin-resistant Staphylococcus aureus (MRSA; n = 759), vancomycin-resistant Enterococcus faecium (VRE; n = 191), extended-spectrum β-lactamase (ESBL)-producing Escherichia coli (n = 602), ESBL-producing Klebsiella pneumoniae (n = 736), and Acinetobacter baumannii (n = 726) that had been collected from patients treated between 2008 and 2010 at 20 hospitals in Taiwan. MICs and inhibition zone diameters were interpreted according to the currently recommended U.S. Food and Drug Administration (FDA) criteria and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. The MIC90 values of tigecycline against MRSA, VRE, ESBL-producing E. coli, ESBL-producing K. pneumoniae, and A. baumannii were 0.5, 0.125, 0.5, 2, and 8 µg/mL, respectively. The total error rates between the two methods using the FDA criteria were high: 38.4% for ESBL-producing K. pneumoniae and 33.8% for A. baumannii. Using the EUCAST criteria, the total error rate was also high (54.6%) for A. baumannii isolates. The total error rates between these two methods were <5% for MRSA, VRE, and ESBL-producing E. coli. For routine susceptibility testing of ESBL-producing K. pneumoniae and A. baumannii against tigecycline, the broth microdilution method should be used because of the poor correlation of results between these two methods.

Antibiotic resistance among clinical bacterial isolates is of major concern worldwide (1). The discovery of new antibiotics has lagged behind the demand for the coverage of emerging clinical multidrug-resistant (MDR) bacterial isolates (18, 21). Clinicians, therefore, are often forced to use agents that are inherently toxic or drugs for which there are no robust data regarding appropriate antibiotic dosing and therapeutic duration to treat patients with infections caused by MDR bacteria (1). Tigecycline, a minocycline derivative with potent activity against a wide range of MDR Gram-positive cocci and Gram-negative rods (15–17, 23), should be highly valued. However, it is very important to ensure that the prescribed antimicrobial is active in vitro against etiologic bacteria, especially when the pathogens that often cause infections in immunocompromised and critically ill patients are MDR (12, 13).
Antibiotic susceptibility can be tested using either a dilution or a diffusion method. A number of such systems are commercially available; the major challenge, however, lies in the inflexibility of standard panels to test the *in vitro* susceptibilities of various bacteria to different antimicrobials (24). Disk diffusion susceptibility testing has been widely used in most clinical laboratories, even though many of those laboratories may be able to purchase automated or semiautomated systems for susceptibility testing. Despite the potential role played by tigecycline in the treatment of infections due to MDR bacteria, little is known about the interchangeability of the results of the disk diffusion and broth microdilution methods in testing the susceptibility of these MDR microbes to tigecycline.

This study compared the *in vitro* susceptibility of various MDR isolates to tigecycline measured by the disk diffusion method with the susceptibility of those isolates measured by the broth microdilution method. The MDR isolates included methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, ESBL-producing *Klebsiella pneumoniae*, and *Acinetobacter baumannii* isolates that had been collected from clinical specimens (blood, respiratory secretion, pus, or urine) during the period from 2008 to 2010. These clinical isolates were part of the bacterial collection in the Tigecycline *In Vitro* Surveillance in Taiwan (TIST) study, a program that commenced in 2006 to longitudinally monitor the *in vitro* activity of tigecycline against a variety of clinical bacteria in Taiwan (10).

### MATERIALS AND METHODS

**Bacterial isolates.** A total of 3,014 isolates consisting of 759 MRSA, 191 VRE, 602 ESBL-producing *E. coli*, 736 ESBL-producing *K. pneumoniae*, and 726 *A. baumannii* isolates were included in this study. The isolates were collected from patients in 20 hospitals, including district hospitals and medical centers, distributed throughout Taiwan. The isolates of those institutions ranged from 450 to 2,500 beds. All isolates were judged to be clinically significant by an infectious disease physician at the collecting hospital. To avoid duplication, each bacterial species was collected only once from an individual patient. Clinical isolates were identified by conventional methods (8, 11, 19, 20) or the automated ID 32GN system (Vitek Systems, bioMérieux, Hazelwood, MO), or both. ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* isolates were identified as recommended by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards [NCCLS]) (4). All *A. baumannii* isolates studied were considered multidrug resistant, as they were resistant to ≥3 of the following antimicrobial agents: ampicillin-sulbactam, aztreonam, cefazidime, ciprofloxacin, gentamicin, imipenem, and piperacillin. The collected isolates were stored at −70°C in skim milk or Trypsinase soy agar supplemented with 15% glycerol. Bacterial identifications were eventually confirmed at the central laboratory at Taiwan National University Hospital, where disk diffusion testing and broth microdilution tigecycline susceptibility testing were carried out. This study was conducted with the ethical approval by the National Taiwan University Hospital Institutional Review Board (NTUH-IRB 9561799108).

**Antimicrobial susceptibility testing.** Susceptibility to tigecycline was tested by the disk diffusion and broth microdilution methods as recommended by the CLSI (2, 3). Standard powder of tigecycline was obtained from Pfizer Inc., NY, for broth microdilution testing. Tigecycline (15 μg per disk) was used for disk diffusion susceptibility testing. Mueller-Hinton agar and broth (BBL Microbiology Systems, Cockeysville, MD) for susceptibility testing were freshly prepared and then used within 12 h (10). Quality control strains, including *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *E. coli* ATCC 25922, were used for susceptibility testing as necessary. Interpretations of the MICs in the broth microdilution method and the diameters of the inhibitory zone in the disk diffusion testing method were based on the criteria proposed by the U.S. Food and Drug Administration (FDA) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), as summarized in Table 1 (6, 16, 17, 23). Some of the results of broth microdilution tests of tigecycline susceptibility against these bacteria obtained in this work will be published elsewhere (Y. H. Chen et al., submitted for publication).

**Agreement assessment of tigecycline susceptibility determined by the disk diffusion and broth microdilution methods.** Agreement was defined as an identical result obtained by both the disk diffusion and broth microdilution tests. Nonagreement referred to errors, which were categorized as very major errors (VMEs), major errors (MaEs), and minor errors (MiEs). Using results of broth microdilution as standards, a VME referred to a false-resistant result by the disk diffusion test, while an MIE referred to a false-susceptible result by the disk diffusion test. An MiE referred to an intermediate result by the disk diffusion test or the broth microdilution test. Acceptable intermethod errors rates were ≤3% for VMEs, ≤5% for MaEs, and ≤10% for MiEs (5). Accurate detection of drug resistance by disk diffusion testing is essential. All MDR isolates were subjected to disk diffusion and broth microdilution methods to interchangeability for determination of the susceptibility of the tested microbes against tigecycline.

### RESULTS

**Tigecycline susceptibilities.** Remarkably high tigecycline susceptibility rates (≥95%) were found among the MRSA, VRE, and ESBL-producing *E. coli* isolates in the broth microdilution testing methods on the basis of both FDA and EUCAST interpretive criteria, among the ESBL-producing *K. pneumoniae* isolates in the broth microdilution method on the basis of FDA interpretive criteria, and among the MRSA, VRE, and ESBL-producing *E. coli* isolates that had been collected from clinical specimens (blood, respiratory secretion, pus, or urine) during the period from March 2012 Volume 56 Number 3 aac.asm.org 1415

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**TABLE 1 Interpretive MIC and disk diffusion interpretive criteria for Gram-positive and Gram-negative bacteria applied in this study**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>MIC (μg/ml)</th>
<th>Diam (mm) by disk diffusion method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>MRSA</td>
<td>≤0.5—0.5</td>
<td>—</td>
</tr>
<tr>
<td>VRE</td>
<td>≤0.25/0.25</td>
<td>—/0.5</td>
</tr>
</tbody>
</table>

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References:

(1) The interpretation MICs in the broth microdilution testing and the diameters of the inhibitory zone in the disk diffusion testing were based on the criteria proposed by the U.S. Food and Drug Administration (FDA)/European Committee on Antimicrobial Susceptibility Testing-2011 (EUCAST-2011) (6, 16, 17, 23).

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(2) Interpretive criteria were not established.

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(3) Interpretive criteria were not established.
isolates in the disk diffusion method on the basis of FDA and EUCAST interpretive criteria (Table 2).

Of note, using the FDA interpretive criteria, a high tigecycline susceptibility rate of 94.6% in the broth microdilution testing method and a low tigecycline susceptibility rate of 69.9% in the disk diffusion testing method were found among the ESBL-producing K. pneumoniae isolates (Table 2). Among the A. baumannii isolates, 68.2% were found to be susceptible to tigecycline by the broth microdilution method and 71.7% were found to be susceptible to tigecycline by the disk diffusion method on the basis of FDA interpretive criteria; however, using the EUCAST interpretive criteria, only 35% of the isolates were susceptible to tigecycline by both the disk diffusion and broth microdilution methods (Table 2).

Discrepancies between results of disk diffusion testing and broth microdilution testing. The total error rates of the disk diffusion method in comparison with the broth microdilution method using the FDA criteria were high: 38.4% for ESBL-producing K. pneumoniae isolates (Table 2). Among the A. baumannii isolates, 68.2% were found to be susceptible to tigecycline by the broth microdilution method and 71.7% were found to be susceptible to tigecycline by the disk diffusion method on the basis of FDA interpretive criteria; however, using the EUCAST interpretive criteria, only 35% of the isolates were susceptible to tigecycline by both the disk diffusion and broth microdilution methods (Table 2).

### DISCUSSION

The rates of susceptibility of ESBL-producing K. pneumoniae isolates and A. baumannii isolates to tigecycline differed markedly between the FDA and EUCAST criteria. The CLSI and FDA criteria are based on clinical and bacteriological response rates in conjunction with population distributions and pharmacokinetics/pharmacodynamics to establish breakpoints in order to provide the best correlation between the in vitro test and clinical results (24). There are, however, a few breakpoint discrepancies between the CLSI and the FDA criteria (24). EUCAST takes a different approach to establishing susceptibility breakpoints by placing greater emphasis on the detection of emerging resistance through examination of microorganism population distributions (24).

On the basis of FDA interpretive susceptibility criteria, the lack of interchangeability between the disk diffusion method and broth microdilution method for tigecycline against our ESBL-producing K. pneumoniae isolates was mainly due to the high percentage of MiEs (26.5%); of note, there was a marked difference in the rate of susceptibility to tigecycline among ESBL-producing K. pneumoniae isolates (69.9% versus 96.3%) between the disk diffusion and broth microdilution methods. Taken together, these data indicate that the disk diffusion method for tigecycline susceptibility testing based on the FDA interpretive susceptibility criteria may underestimate the rate of susceptibility to tigecycline among the ESBL-producing K. pneumoniae isolates. On the basis of our findings, we suggest that when the disk diffusion method shows ESBL-producing K. pneumoniae isolates susceptible to tigecycline on the basis of the FDA interpretation-based category, tigecycline is the appropriate antibiotic for treating infections caused by these pathogens.

Given that previously published and current results on the tigecycline susceptibility of A. baumannii isolates differ markedly between the disk diffusion method and the broth microdilution method, as well as between the agar-diffusion-based Etest and the broth microdilution method (14, 16), it is not surprising that the

### TABLE 2 Susceptibilities to tigecycline determined by broth microdilution and disk diffusion methods

<table>
<thead>
<tr>
<th>Bacterium (no. of isolates)</th>
<th>MIC (µg/ml)</th>
<th>% susceptibility by broth microdilution method/disk diffusion method by use of interpretive criteria of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FDA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>MRSA (759)</td>
<td>0.03–1</td>
<td>0.125</td>
</tr>
<tr>
<td>VRE (191)</td>
<td>0.016–1</td>
<td>0.03</td>
</tr>
<tr>
<td>ESBL-producing E. coli (602)</td>
<td>0.125–8</td>
<td>0.25</td>
</tr>
<tr>
<td>ESBL-producing K. pneumoniae (736)</td>
<td>0.125–8</td>
<td>0.5</td>
</tr>
<tr>
<td>A. baumannii (726)</td>
<td>0.06–64</td>
<td>2</td>
</tr>
</tbody>
</table>

a MRSA, methicillin-resistant Staphylococcus aureus; VRE, vancomycin-resistant Enterococcus faecium.

b S, susceptible; I, intermediate; R, resistant; FDA, U.S. Food and Drug Administration; EUCAST, European Committee on Antimicrobial Susceptibility Testing; —, interpretive criteria were not established.

### TABLE 3 Error rates of tigecycline susceptibilities by disk diffusion method in comparison with broth microdilution method

<table>
<thead>
<tr>
<th>Bacterium (no. of isolates)</th>
<th>% isolates with indicated error on the basis of interpretive criteria of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FDA</td>
</tr>
<tr>
<td></td>
<td>VME</td>
</tr>
<tr>
<td>MRSA (759)</td>
<td>—</td>
</tr>
<tr>
<td>VRE (191)</td>
<td>—</td>
</tr>
<tr>
<td>ESBL-producing E. coli (602)</td>
<td>0</td>
</tr>
<tr>
<td>ESBL-producing K. pneumoniae (736)</td>
<td>0</td>
</tr>
<tr>
<td>A. baumannii (726)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

a MRSA, methicillin-resistant Staphylococcus aureus; VRE, vancomycin-resistant Enterococcus faecium; VME, very major error; MaE, major error; MiE, minor error; FDA, U.S. Food and Drug Administration; EUCAST, European Committee on Antimicrobial Susceptibility Testing; —, interpretive criteria were not established.
results from the disk diffusion and broth microdilution susceptibility testing methods are not interchangeable. The variability in magnesium and oxygen contents in the susceptibility testing media has been shown to interfere with the results of tigecycline susceptibility testing against A. baumannii isolates (9, 22).

In summary, we found that both the disk diffusion method and broth microdilution method showed high rates of tigecycline susceptibility among MRSA, VRE, and ESBL-producing E. coli isolates on the basis of both FDA and EUCAST interpretive criteria and among ESBL-producing K. pneumoniae isolates on the basis of FDA interpretive criteria. Our data suggest that the disk diffusion method, with its inherent flexibility in antibiotic selection and low cost, may be used as a substitute for the broth microdilution method in tigecycline susceptibility testing against MRSA, VRE, and ESBL-producing E. coli isolates based on both FDA and EUCAST interpretive criteria. For routine susceptibility testing of tigecycline against ESBL-producing K. pneumoniae and A. baumannii, the broth microdilution method and not the disk diffusion method should be used due to the poor correlation of results between these two methods.

ACKNOWLEDGMENT

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REFERENCES

7. Reference deleted.