Influence of testing methodology on the tigecycline activity profile against presumably tigecycline-non-susceptible Acinetobacter spp.

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Objectives: To compare the tigecycline activity profile against Acinetobacter spp. by Etest versus broth microdilution in isolates with high Etest MIC.

Methods: Acinetobacter spp. isolates with tigecycline MICs of ≥0.5 mg/L determined by commercially developed Etests strips (January 2006 to July 2007) in five Spanish hospitals were considered. Values were rounded to the nearest upper double-dilution. Susceptibility by broth microdilution following CLSI (formerly NCCLS) recommendations, as the reference method, was determined in a central laboratory. BSAC breakpoints were used: susceptible ≤1 mg/L; intermediate = 2 mg/L; and resistant ≥2 mg/L.

Results: One hundred and forty-eight isolates were collected: 12 isolates with a tigecycline Etest MIC of 0.5 mg/L, 14 with 1 mg/L, 86 with 2 mg/L, 31 with 4 mg/L and 5 with 8 mg/L. Isolates with Etest MICs of 0.5–1 mg/L showed the same values by broth microdilution. Among isolates with Etest MICs of 2 mg/L, only 5.8% of strains showed the same value by both methods (88.4% showed values that were one or two dilutions lower by microdilution). None of the 36 isolates with Etest MICs of 4–8 mg/L showed the same value by both methods, with values at least two dilutions lower by microdilution. Weak correlation (R=0.238; P≤0.001) was found between both methods. All 26 Etest susceptible isolates, 80/86 (93.0%) Etest intermediate and 32/36 (88.9%) Etest resistant strains were susceptible by microdilution.

Conclusions: Caution should be taken in interpreting Etest MICs of ≥2 mg/L for Acinetobacter spp. since strains with Etest MICs of 2–4 mg/L are susceptible when tested by microdilution. False non-susceptibility by Etest may exclude tigecycline as a therapeutic option in a field where multiresistance is the rule.

Keywords: Etest, broth microdilution, BSAC breakpoints

Introduction

A recent review reported a favourable clinical response for Acinetobacter spp. infections treated with tigecycline.1 However, the outcome may be compromised in patients infected by isolates with a decreased susceptibility to tigecycline, as reported in one of the series included in the review.1 As a new agent, surveillance of susceptibility to tigecycline is critical to detect changes in its activity profile, which depends on the medium used for susceptibility testing, testing methodology and
on the MIC normal distribution relative to the breakpoint value. This may be critical in an area where multiresistance is the rule as occurs in Acinetobacter spp. in Spain, which exhibits high resistance rates of ~40% to carbapenems and >75% to quinolones, ceftazidime and gentamicin.

With respect to the medium used for susceptibility testing, differences in MIC values have been reported when determinations are performed using some commercial Mueller-Hinton media with elevated manganese concentrations. This fact may increase the reported difference in the MIC values obtained when using different testing methodologies, with higher MIC values (one to two dilutions) in determinations by Etest versus broth microdilution. However, it is unknown if this dichotomy occurs in all values of the tigecycline MIC range. Lastly, for Acinetobacter spp. there are no tigecycline breakpoints defined by the Food and Drug Administration (FDA) or the European Society of Clinical Microbiology and Infectious Diseases (EUCAST), but values of ≤1, 2 and >2 mg/L should be considered for the susceptible, intermediate and resistant categories according to the BSAC.

The aim of this study was to compare the activity profile of tigecycline against Acinetobacter spp. when determined by Etest versus broth microdilution in isolates exhibiting a high Etest MIC of tigecycline.

Materials and methods

Strains

Microbiology departments in five Spanish hospitals were contacted to prospectively obtain Acinetobacter spp. isolates with tigecycline MICs of ≥0.5 mg/L, determined by Etest, between January 2006 and July 2007. Isolates were frozen and sent to the study’s central laboratory at Laboratories International for Microbiology Studies, International Health Management Associates Inc., for determination of tigecycline susceptibility by broth microdilution.

Susceptibility testing

In the hospitals, susceptibility to tigecycline was determined using commercially developed tigecycline Etest strips (AB Biodisk, Solna, Sweden) according to the manufacturer’s instructions. Briefly, an inoculum suspension with a turbidity equivalent to that of a 0.5 McFarland standard was prepared by suspending isolated colonies in 0.9% saline. A sterile cotton swab dipped into the suspension was used to evenly streak Mueller-Hinton agar (MHA) from bioMérieux (Marcy l’Étoile, France) in four centres and MHA from Oxoid for 16 isolates (one centre). The Etest tigecycline gradient strip was applied to the agar surfaces and plates were incubated in ambient air at Hampshire, UK) in one centre. The Etest tigecycline gradient strip was used to determine the MIC of tigecycline.

Ellipse intersected the MIC on the Etest gradient strip. Values read were rounded to the nearest upper double-dilution value.

Materials and methods

Results

One hundred and forty-eight isolates with the requested Etest tigecycline MIC of ≥0.5 mg/L were collected. MHA bioMérieux was used for Etest MIC determination for 132 isolates (four centres) and MHA from Oxoid for 16 isolates (one centre). Twelve isolates had a tigecycline MIC of 0.5 mg/L, 14 an MIC of 1 mg/L, 86 an MIC of 2 mg/L, 31 an MIC of 4 mg/L and 5 an MIC of 8 mg/L. According to BSAC breakpoints, 26 isolates were susceptible (MIC ≤1 mg/L), 86 were intermediate (MIC = 2 mg/L) and 36 were resistant (MIC > 2 mg/L) to tigecycline.

Table 1 shows the correlation between Etest and broth microdilution. A weak correlation (R = 0.238; P ≤ 0.001) was found between both methods when considering the 148 isolates tested. Isolates with an Etest MIC of 0.5–1 mg/L (susceptible) showed the same MIC when susceptibility was determined by broth microdilution, except in one case where the MIC by broth microdilution was one dilution lower. Discrepancies arose in intermediate isolates (MIC = 2 mg/L) where only 5.8% of strains showed the same MIC value by both methods, while 88.4% showed MIC values that were one or two dilutions lower by microdilution. All four isolates showing a microdilution MIC of 0.12 mg/L (four dilutions lower than the Etest MIC of 2 mg/L) came from the hospital that used MHA from Oxoid as the Etest medium. Discrepancies between Etest and microdilution were greater in strains resistant by Etest (MIC = 4–8 mg/L) since none of the 36 isolates tested showed the same MIC value by both methods, and all but one isolate showed values by microdilution at least two dilutions lower than those by Etest.

Table 2 shows the influence of these discrepancies in susceptibility and resistance rates of tigecycline by both methods according to BSAC breakpoints. While all 26 susceptible strains by Etest (MICs of 0.5–1 mg/L) were also susceptible by broth microdilution, 80/86 (93.0%) strains intermediate by Etest (MIC = 2 mg/L) were susceptible by microdilution and 32/36 (88.9%) strains resistant by Etest (MIC = 4–8 mg/L) were susceptible by microdilution.

Discussion

An Etest has recently been developed for the susceptibility testing of tigecycline. For most evaluated pathogens, Etest MICs tended to be one doubling-dilution higher than broth microdilution MICs and, in general, high correlation coefficients were obtained for Enterobacteriaceae with a negligible number of major errors (false non-susceptibility by Etest). In Acinetobacter spp., due to the absence of CLSI breakpoints, false non-susceptibility by Etest was not explored.

The correlation between Etest and broth microdilution was high in a previous study by Pillar et al., with a correlation coefficient of 0.865, a value much higher than the one obtained in the present study (0.238). This difference is probably due to the fact that the previous study was performed with non-selected strains, including a high number of strains with an Etest MIC ≤1 mg/L (susceptible following BSAC breakpoints), whereas in the present study the majority of strains (122/148; 82.4%) showed MICs of ≥2 mg/L (non-susceptible), as centres were asked to send isolates with MICs of ≥0.5 mg/L. In this sense, discrepancies between both methods occurred in intermediate (MIC values 1–2 dilutions lower for broth microdilution) and
Tigecycline Etest versus microdilution and *Acinetobacter* spp.

Table 1. Correlation between Etest and broth microdilution in 148 *Acinetobacter* spp. isolates with Etest MIC of ≥0.5 mg/L.

<table>
<thead>
<tr>
<th>Strains with tigecycline Etest MIC (mg/L) of</th>
<th>Microdilution versus Etest n (%)</th>
<th>MIC variation (log₂ dilutions)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>−4</td>
</tr>
<tr>
<td>0.5</td>
<td>12</td>
<td>12 (100)</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>13 (92.9)</td>
</tr>
<tr>
<td>2</td>
<td>86</td>
<td>5 (5.8)</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>5 (16.1)</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>5 (3.4)</td>
</tr>
</tbody>
</table>

*A negative number indicates the number of dilutions that the MIC determined by broth microdilution was lower than the MIC determined by Etest. A positive number indicates the number of dilutions that the MIC determined by broth microdilution was higher than the MIC determined by Etest. ‘0’ indicates identical MIC value by both methods.

Table 2. Susceptibility and resistance rates by Etest and microdilution according to BSAC breakpoints for 148 *Acinetobacter* spp. isolates with Etest MIC of ≥0.5 mg/L.

<table>
<thead>
<tr>
<th>Etest</th>
<th>Broth microdilution</th>
<th>n</th>
<th>MIC</th>
<th>MIC⁵₀</th>
<th>MIC⁹₀</th>
<th>range</th>
<th>%S</th>
<th>%I</th>
<th>%R</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.5</td>
<td>12</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5–0.5</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>0.5–1</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>2</td>
<td>86</td>
<td>1</td>
<td>1</td>
<td>0.12–4</td>
<td>93</td>
<td>5.8</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>4</td>
<td>31</td>
<td>1</td>
<td>1</td>
<td>0.5–1</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>0.5–4</td>
<td>20</td>
<td>60</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*S, susceptible (<1 mg/L); I, intermediate (=2 mg/L); R, resistant (>2 mg/L).

resistant (MIC values 2–3 dilutions lower for broth microdilution) isolates by Etest, while the same MIC value (all but one isolate) was obtained by both methods for tigecycline-susceptible strains (MIC 0.5–1 mg/L) by Etest. As the values of MIC determined by Etest increased, discrepancies between both methods increased, but always in the non-susceptibility Etest MIC range.

Considering the reference method of broth microdilution and applying the BSAC susceptibility breakpoint, high percentages of major errors (false non-susceptibility by Etest) were found since 93% of intermediate and 88.9% of strains resistant by Etest were susceptible when tested by broth microdilution. No major errors were found in the susceptible category, with all strains being susceptible by both methods.

During nationwide surveillance in Spain, tigecycline MIC values for the isolates tested were higher in one centre (University Hospital Marques de Valdecilla) than in others, probably due to the source of the MHA medium in that centre (Merck). The hypothesis of MIC variation depending on the different compositions of Mueller-Hinton medium was corroborated and higher resistance rates (as defined by the BSAC resistance breakpoint: >2 mg/L) were found when using Merck versus BBL or Biomedics. Tigecycline MICs determined in MHA containing low manganese concentration may be more clinically relevant due to the low manganese concentrations in human serum (far from the high concentrations in Merck’s MHA).

The major finding of the present study was that discrepancies between Etest and microdilution were clustered in tigecycline-intermediate or -resistant strains (when MICs were determined by Etest). Caution should be taken in interpreting Etest MICs of ≥2 mg/L since strains with Etest MICs of ≥2–4 mg/L are susceptible when tested by microdilution, and non-susceptibility by broth microdilution is only clearly clustered in isolates with very high Etest MICs (8 mg/L). False non-susceptibility by Etest may exclude tigecycline as a therapeutic option in a field where multiresistance is the rule. Further studies are needed to define the most adequate MHA medium (with adequate manganese concentration) in tigecycline susceptibility testing for *Acinetobacter* spp. with diffusion methods.

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Transparency declarations

N.G.-E. is an employee of Wyeth Farma S.A., Madrid, Spain, not owning stock or options in the company. C.G.-R. was an employee of Wyeth Farma S.A. at the time of the study, not owning stock or options in the company. All other authors: none to declare.

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