Impact of carbon dioxide on the susceptibility of key respiratory tract pathogens to telithromycin and azithromycin

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Objectives: To determine the quantitative differences in telithromycin and azithromycin MIC values against Streptococcus pneumoniae, Haemophilus influenzae and Streptococcus pyogenes obtained using two recommended and commonly used methodologies: CLSI reference standard broth microdilution in ambient air and Etest® concentration gradient in CO2.

Methods: Four hundred clinical isolates (S. pneumoniae, n=200; H. influenzae, n=100; S. pyogenes, n=100) were evaluated in seven independent laboratories. Telithromycin and azithromycin MICs were determined using CLSI broth microdilution panels incubated in ambient air and Etest® strips incubated in CO2. Standard quality control reference strains—S. pneumoniae ATCC 49619 (n=10) and H. influenzae ATCC 49247 (n=10)—were also tested.

Results: Telithromycin and azithromycin Etest® MICs in CO2 were elevated for all organisms when compared with values obtained using broth microdilution in ambient air. Telithromycin geometric mean MIC values increased in CO2 by 2.05, 1.00 and 1.78 log2 dilutions for S. pneumoniae, H. influenzae and S. pyogenes, respectively. The corresponding values for azithromycin were 2.54, 1.21 and 3.0 log2 dilutions, respectively.

Conclusions: Telithromycin MICs measured using Etest® in CO2 are consistently elevated compared with those generated by CLSI broth microdilution measured in ambient air. These findings indicate that Etest® should not be routinely used for the determination of telithromycin MICs against S. pneumoniae, H. influenzae and S. pyogenes, unless appropriate corrective factors are applied before reporting MICs or applying interpretive susceptibilities. Based on results from this study, Etest® MIC breakpoints and quality control ranges are proposed.

Keywords: ketolides, S. pneumoniae, H. influenzae, S. pyogenes, susceptibility testing

Introduction

Telithromycin is the first ketolide antibiotic to be approved for clinical use in the treatment of respiratory tract infections. It demonstrates good in vitro activity against common respiratory pathogens such as Streptococcus pneumoniae, Haemophilus influenzae and Streptococcus pyogenes, including pneumococci resistant to penicillin and/or macrolides.

The antibacterial activity of telithromycin has been evaluated extensively in vitro. Quality control values and susceptibility breakpoints have been established for MICs using standardized microbiological procedures established by the US Food and Drug Administration (FDA) and the CLSI. According to these protocols, MICs are determined using broth microdilution panels incubated in ambient air.

The Etest® (AB Biodisk, Solna, Sweden) is a popular commercial, non-reference antibacterial susceptibility testing product used to determine MIC values. It is notable that fastidious organisms, such as those frequently implicated in lower respiratory tract infections, require incubation in carbon dioxide (CO2) to promote growth and facilitate the determination of MICs by this concentration gradient methodology. However, incubation in CO2 has been reported to result in falsely elevated MIC values for certain antibacterials (the macrolides and azalides), with MICs obtained in CO2 typically 1–3 log2 dilutions higher than those obtained by broth microdilution in ambient air. It has been suggested that the increase in macrolide or azalide MICs induced by CO2 may be attributable to acidification of the test medium. If the reduction in pH seen during incubation in CO2 is compensated for, macrolide MIC values obtained using the Etest®...
Impact of CO₂ on telithromycin MIC

are comparable to those generated during incubation in ambient air.⁶

Since telithromycin is structurally related to the macrolides, it seems reasonable to assume that telithromycin MICs may also be influenced by the presence of CO₂. This study was undertaken to determine the quantitative differences in telithromycin and azithromycin MIC values against S. pneumoniae, H. influenzae and S. pyogenes obtained using the Etest® in CO₂ compared with MICs obtained via broth microdilution methodology in ambient air.

Materials and methods

Isolates

A total of 400 banked clinical isolates were evaluated in this study, comprising 200 strains of S. pneumoniae, 100 strains of H. influenzae and 100 strains of S. pyogenes. Isolates were supplied by Laboratories International for Microbiology Studies, Inc. (Schaumburg, IL, USA) and no isolate was more than 18 months old. All isolates were stored at −70°C and double-passed prior to testing.

Study design

The antimicrobial susceptibility testing protocol was designed to emulate, as closely as possible, the M23 format recommended by the CLSI Subcommittee on Antimicrobial Susceptibility Testing for the establishment of quality control ranges for antimicrobials. This protocol is designed to minimize biases that may exist between laboratories.

Seven independent reference laboratories in Canada and the USA tested each strain twice—one by broth microdilution in ambient air and once using Etest® strips incubated in 5% CO₂—over a period of 10 days in a predetermined order according to a standardized protocol. Each of the seven reference laboratories tested each strain using identical lot numbers of MIC panels, Etest® strips and reagents. All laboratories followed CLSI guidelines for testing of fastidious organisms in a CO₂ environment. Standard quality control strains were also tested at all laboratories: namely, S. pneumoniae ATCC 49619 (n = 10) and H. influenzae ATCC 49247 (n = 10). Azithromycin was selected for use as a positive control in this study.

Antimicrobial susceptibility testing

Susceptibility testing was performed using broth microdilution panels (PML Microbiologicals, Wilsonville, OR, USA) tested in ambient air. Panels were stored at −70°C and thawed at room temperature before use. Etest® strips (AB Biodisk, Solna, Sweden) were tested in an atmosphere of 5% CO₂ according to CLSI guidelines and Etest® recommendations.²,⁵

Isolates tested in the study were subcultured in appropriate media before susceptibility testing. A common inoculum for the panels and Etest® strips was made for each strain by overnight culture of the organism using 5.0 mL of Mueller–Hinton broth or 0.9% physiological saline to a turbidity equal to a 0.5 McFarland standard. Periodic colony counts were performed to ensure inoculum uniformity.

Broth microdilution panels contained either lysed horse blood-supplemented, cation-adjusted Mueller–Hinton broth recommended specifically for MIC testing of streptococci or Haemophilus Test Media (HTM) for MIC testing of H. influenzae. Panels were incubated in ambient air at 35°C for 20–24 h. Broth microdilution MICs were determined as the first well containing the lowest concentration of antibiotic with no readily visible growth or haze, as detected by the unaided eye.

Plates prepared from a common lot of agar were used throughout the study to perform susceptibility testing by Etest®. Each plate was provided with 150 mm plates containing Mueller–Hinton agar and 5% defibrinated sheep blood (MHASB) for growing S. pneumoniae and S. pyogenes, and with 150 mm HTM agar plates for growing each strain of H. influenzae. All plates were incubated at 35°C in 5% CO₂ for 20–24 h. Etest® end points were read at the point of complete inhibition of all growth—including hazes and isolated colonies—as stated in the Etest® technical guide.⁶

Quality control of panels and plates was performed using ATCC quality control strains on each day of testing at each laboratory according to CLSI guidelines.

Data analysis

All data for broth microdilution and Etest® MICs were collected and analysed by International Health Management Associates, Inc. (Schaumburg, IL, USA).

Results and discussion

A total of 400 isolates were tested at each of the seven laboratories involved in this study. In all, 2769/2800 (99%) of the duplicate tests (broth microdilution and Etest®) were completed (S. pneumoniae, 1385/1400; H. influenzae, 690/700; S. pyogenes, 694/700); 31 tests were excluded from this analysis due to isolates failing to grow in CO₂. The in vitro activities of telithromycin and azithromycin against test strains and ATCC quality control strains (S. pneumoniae and H. influenzae) by CLSI broth microdilution in ambient air and Etest® in 5% CO₂ are shown in Table 1. Telithromycin and azithromycin MICs measured using the Etest® were consistently elevated compared with broth microdilution. Telithromycin geometric mean MIC values increased when determined by Etest® by 2.05, 1.00 and 1.78 log₂ dilutions for S. pneumoniae, H. influenzae and S. pyogenes, respectively. The corresponding values for azithromycin were 2.54, 1.21 and 3.00 log₂ dilutions, respectively.

Comparison of the geometric mean values obtained by broth microdilution and Etest® for the S. pneumoniae quality control reference strain (ATCC 49619) indicated differences of +1.62 and +3.03 log₂ dilutions for telithromycin and azithromycin, respectively (Table 1). Against H. influenzae ATCC 49247, a difference of +1.18 log₂ dilutions was recorded between the telithromycin geometric mean values for broth microdilution and Etest®; for azithromycin, the difference was +1.64 log₂ dilutions.

Telithromycin MICs for S. pyogenes have previously been reported to be increased by up to six doubling dilutions when measured in CO₂ compared with ambient air⁷ and elevated telithromycin MICs against S. pneumoniae and H. influenzae have been reported in the presence of CO₂.² Similarly, azithromycin MICs have been reported to be higher in the presence of CO₂ versus ambient air.⁸ The CO₂-induced increase in telithromycin MICs may result in isolates being falsely classified as having reduced susceptibility to telithromycin when using Etest® methodology. Walsh and colleagues reported that 5% of strains of S. pneumoniae and 4% of H. influenzae strains changed
classification from telithromycin susceptible to non-susceptible following incubation in CO₂. However, the clinical significance of this loss of in vitro activity of telithromycin following incubation in CO₂ remains to be determined.

These findings highlight the need for standardization of telithromycin testing methods and interpretative criteria to ensure the optimum use of this ketolide antibacterial. Suggested Etest MIC breakpoints for telithromycin against S. pneumoniae, H. influenzae and S. pyogenes are proposed in this paper based on the data generated in this study (Table 2). However, further validation is clearly required in other studies before definitive recommendations can be made.

In summary, results of this study show telithromycin MICs to be elevated when measured in the presence of CO₂ compared with those obtained by testing in ambient air. These data support a +2 log₂ correction for reporting Etest results when testing telithromycin against S. pneumoniae and a +1 log₂ correction for CLSI interpretive criteria when testing telithromycin against H. influenzae using the Etest methodology in CO₂. As no CLSI-approved breakpoints are available for telithromycin against S. pyogenes, we used interpretive criteria published by the French Society of Microbiology and the results from this study suggest that a +1 log₂ correction should be applied for reporting Etest results. If Etest methodology is used to determine telithromycin MICs for these pathogens, the appropriate log₂ correction factors should be applied by the laboratory and only the resulting susceptibilities should be reported to clinical physicians in order to ensure accurate interpretation of telithromycin susceptibility and thus allow optimum use of this ketolide antibacterial in the clinical setting.

Acknowledgements
This study was funded by Sanofi-Aventis, Bridgewater, NJ, USA.

Table 1. In vitro activity of telithromycin and azithromycin against 400 clinical isolates of Streptococcus pneumoniae, Haemophilus influenzae and Streptococcus pyogenes comparing broth microdilution MICs with Etest MICs

<table>
<thead>
<tr>
<th>Organism</th>
<th>Broth microdilution panel in ambient air</th>
<th>Etest® in 5% CO₂</th>
<th>Log₂ dilution differences: Etest® versus broth microdilution</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MIC₅₀</td>
<td>MIC₉₀ geometric mean</td>
<td>MIC₅₀</td>
</tr>
<tr>
<td>S. pneumoniae (n = 1385)</td>
<td>telithromycin</td>
<td>0.015</td>
<td>0.5</td>
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<tr>
<td></td>
<td>azithromycin</td>
<td>1</td>
<td>128</td>
</tr>
<tr>
<td>H. influenzae (n = 690)</td>
<td>telithromycin</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>azithromycin</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>S. pyogenes (n = 694)</td>
<td>telithromycin</td>
<td>0.015</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>azithromycin</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>S. pneumoniae ATCC 49619 (n = 418)</td>
<td>telithromycin</td>
<td>0.015</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>azithromycin</td>
<td>0.25</td>
<td>b</td>
</tr>
<tr>
<td>H. influenzae ATCC 49247 (n = 418)</td>
<td>telithromycin</td>
<td>2</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>azithromycin</td>
<td>2</td>
<td>b</td>
</tr>
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</table>

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<tr>
<th>Organism</th>
<th>CLSI interpretive criteriaa</th>
<th>suggested Etest® interpretive criteria</th>
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<td></td>
<td>≤S</td>
<td>I</td>
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<tr>
<td>S. pneumoniae</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>4</td>
<td>8</td>
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<tr>
<td>S. pyogenes</td>
<td>0.5b</td>
<td>1b</td>
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<table>
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<tr>
<th>ATCC strains</th>
<th>CLSI quality control rangesa</th>
<th>suggested Etest® quality control ranges</th>
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<tbody>
<tr>
<td>S. pneumoniae, 49619</td>
<td>0.004–0.03</td>
<td>0.015–0.12</td>
</tr>
<tr>
<td>H. influenzae, 49247</td>
<td>1–4</td>
<td>4–16</td>
</tr>
</tbody>
</table>

I, intermediate; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.
aBreakpoints and quality control ranges as defined in CLSI document M100-S15 unless otherwise noted.

These findings highlight the need for standardization of telithromycin testing methods and interpretative criteria to ensure the optimum use of this ketolide antibacterial. Suggested Etest MIC breakpoints for telithromycin against S. pneumoniae, H. influenzae and S. pyogenes are proposed in this paper based on the data generated in this study (Table 2). However, further validation is clearly required in other studies before definitive recommendations can be made.

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References


