In Vitro Activities of a New Ketolide, ABT-773, against Multidrug-Resistant Gram-Positive Cocci

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The in vitro activities of ABT-773 were evaluated against 324 strains of gram-positive bacteria, including multidrug-resistant Staphylococcus spp. and Enterococcus spp. ABT-773 had lower MIC ranges, MICs at which 50% of isolates are inhibited (MIC50), and MIC90s than erythromycin or clindamycin for almost all isolates tested. The MICs of ABT-773 were also lower than those of quinupristin-dalfopristin (Q-D) for methicillin-susceptible Staphylococcus aureus, Rhodococcus spp., and Streptococcus spp., while the MICs of Q-D were lower than those of ABT-773 for methicillin-resistant S. aureus and Enterococcus faecium, including vancomycin-resistant isolates.

The emergence of resistance in gram-positive bacteria and other bacteria to currently available antimicrobials such as vancomycin (VAN), macrolides, beta-lactam antibiotics, and quinolones (5, 9, 10) and the reported isolation of VAN-intermediate (VANi) Staphylococcus aureus strains (3, 6) have resulted in a clear need to discover new antibiotics. Ketolides have been reported to be active against resistant gram-positive bacteria including erythromycin-resistant (ERYr) and penicillin-resistant Streptococcus pneumoniae strains (1, 4). ABT-773 is a new ketolide which has been reported to have excellent activity against S. pneumoniae, staphylococci, and other respiratory pathogens (2; M. H. Bui, L. S. Almer, D. M. Hensey, Z. Ma, Y. S. Or, A. M. Nilius, and R. K. Flamm, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2136, 1999). However, its activity against multidrug-resistant enterococci has not been well represented in those studies. In the present study, we determined the in vitro activities of ABT-773 by the agar dilution and the broth microdilution methods and compared these to the activities of ERY, clindamycin (CLI), and quinupristin-dalfopristin (Q-D) against 324 gram-positive organisms (including 156 enterococci) that display various degrees of resistance to antimicrobial agents.

(This work was presented in part at the 5th International Conference on the Macrolides, Azalides, Streptogramins, Ketolides and Oxazolidinones, Seville, Spain, 2000, abstr. 2.14.)

The organisms tested in the present study included isolates of Enterococcus spp. (n = 156; beta-lactamase producers, high-level gentamicin and VAN resistant), Staphylococcus spp. (n = 34; methicillin-susceptible S. aureus [MSSA] and methicillin-resistant S. aureus [MRSA]), Streptococcus spp. (n = 102), Rhodococcus spp. (n = 14), Pediococcus spp. (n = 6), Leuconostoc spp. (n = 7), Lactobacillus spp. (n = 3), and Corynebacterium spp. (n = 2) which were collected between 1980 and 1999 from the United States, Argentina, Belgium, Chile, and Thailand. These isolates were identified to the species level by defined biochemical tests, with commercially available Analytab Products strips (bioMérieux Vitek, Inc. Hazelwood, Mo.), and/or by using species-specific DNA gene probes (15). The ketolide compound ABT-773 was obtained from Abbott Laboratories, North Chicago, Ill.; ERY and CLI were obtained from Sigma, St. Louis, Mo.; and Q-D was obtained from Rhône-Poulenc Rorer, Vitry sur Seine, France. The MIC of each antimicrobial agent was determined by following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for antimicrobial susceptibility testing by the agar dilution and broth microdilution methods (11). Mueller-Hinton medium (Becton Dickinson and Company, Cockeysville, Md.) with serial twofold dilutions of antibiotics was used. Incubation was carried out at 35 ± 1°C. The standard reference strains used were Enterococcus faecalis ATCC 29212, S. aureus ATCC 29213, S. pneumoniae ATCC 49619, and Escherichia coli ATCC 25922. The susceptibilities to ERY, CLI, and Q-D were interpreted according to the recommendations of NCCLS (12). The susceptibility breakpoints of ERY (12) for Streptococcus spp. including S. pneumoniae are ≤0.25 µg/ml for susceptibility (ERY1), ≤5 µg/ml for intermediate (ERY2), and ≥1 µg/ml for resistance (ERY3), while for Enterococcus spp. and Staphylococcus spp., these breakpoints are ≤0.5 µg/ml for ERY1, 1 to 4 µg/ml for ERY2, and ≥8 µg/ml for ERY3 (12). The presence of ERY resistance genes erm(B) and mef(A/E) (13, 16) was determined by hybridization of DNA probes with colony lysates under high-stringency conditions (15).

The results of susceptibility testing of 324 organisms with ABT-773 and the other antibiotics tested are summarized in Tables 1 and 2. Among the 19 MSSA isolates, 2 isolates were...
<table>
<thead>
<tr>
<th>Bacterial strains (no. isolates tested) and drug</th>
<th>MIC (μg/ml)</th>
<th>Bacterial strains (no. isolates tested) and drug</th>
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<td>90%</td>
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<tr>
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<td>64</td>
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<tr>
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<td>≤0.007–64</td>
<td>0.25</td>
<td>64</td>
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<tr>
<td>CL1</td>
<td>≤0.007–64</td>
<td>0.25</td>
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</tr>
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<td>64</td>
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<tr>
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<td>0.031</td>
<td>0.25</td>
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<tr>
<td>ERY</td>
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<td>0.031</td>
<td>0.25</td>
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<td>0.25</td>
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<tr>
<td>ERY</td>
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<td>CL1</td>
<td>≤0.007–0.62</td>
<td>0.031</td>
<td>0.25</td>
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</tbody>
</table>

\(^a\) One isolate was ERY\(^r\) and two isolates were ERY\(^i\).
TABLE 2. Susceptibilities of the HLER, *erm*(B)-positive *E. faecalis* and *E. faecium* isolates tested

<table>
<thead>
<tr>
<th>Organism and no. of isolates</th>
<th>MIC (μg/ml)</th>
<th>ABT-773</th>
<th>ERY</th>
<th>CLI</th>
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<tr>
<td><em>E. faecalis</em> (n = 60)</td>
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<tr>
<td>20</td>
<td>0.031–0.5</td>
<td>&gt;512</td>
<td>32–&gt;256&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>16</td>
<td>1–4</td>
<td>&gt;512</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>&gt;8–32</td>
<td>&gt;512</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>≥64</td>
<td>&gt;512</td>
<td>&gt;256</td>
<td></td>
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<tr>
<td><em>E. faecium</em> (n = 54)</td>
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<tr>
<td>4</td>
<td>0.25</td>
<td>&gt;512</td>
<td>4–&gt;256&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>10</td>
<td>1–4</td>
<td>&gt;512</td>
<td>≥256</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>8–32</td>
<td>256–&gt;512</td>
<td>0.125–&gt;256&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>3</td>
<td>&gt;64</td>
<td>&gt;512</td>
<td>&gt;256</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The MICs were <256 μg/ml for only two isolates (MICs for both isolates, 32 μg/ml).
<sup>b</sup> The MIC was <256 μg/ml for only one isolate (MIC, 4 μg/ml).
<sup>c</sup> The MIC was <256 μg/ml for only one isolate (MIC, 0.125 μg/ml).

ERY<sup>a</sup> (MIC of ERY, >512 μg/ml; MIC of CLI, 0.125 μg/ml) and were classified by the double-disk diffusion method (8) as having an inducible macrolide-lincosamide-streptogramin B (iMLS) phenotype. Both of these isolates with the iMLS phenotype were inhibited by ABT-773 at 0.031 to 0.125 μg/ml. For the remaining 17 MSSA isolates, ABT-773 showed 8 times lower MICs at which 50% of isolates are inhibited (MIC<sub>50</sub>) and MIC<sub>90</sub> than ERY and 16 to 32 times lower MIC<sub>50</sub> and MIC<sub>90</sub> than CLI and Q-D. All MRSA isolates used in the present study were resistant to ERY and CLI (ERY and CLI MICs, >512 and >256 μg/ml, respectively), and ABT-773 at concentrations ≤64 μg/ml was unable to inhibit these strains.

Of 92 *E. faecalis* isolates tested, 13 were VAN susceptible (VAN<sup>a</sup>) and ERY<sup>a</sup>, 67 were VAN<sup>n</sup> and ERY<sup>a</sup> or ERY<sup>b</sup>, and 12 were VAN<sup>n</sup> and ERY<sup>n</sup> or ERY<sup>b</sup>. In comparison to ERY, MIC<sub>50</sub> and MIC<sub>90</sub> for *E. faecalis* isolates, ABT-773 MIC<sub>50</sub> and MIC<sub>90</sub> were 64 and 32 times lower for VAN<sup>n</sup> and ERY<sup>n</sup>, respectively; 1,024 and 16 times lower for VAN<sup>n</sup> and ERY<sup>n</sup> or ERY<sup>n</sup> isolates, respectively. All ERY<sup>n</sup> and 13 of 79 ERY<sup>b</sup> or *E. faecalis* isolates were inhibited by ABT-773 at ≤0.062 μg/ml. For all E. faecalis isolates the ABT-773 MIC was >0.062 μg/ml and the ERY MIC was >512 μg/ml. A total of 64 *Enterococcus faecium* isolates (22 VAN<sup>n</sup> isolates and 42 VAN<sup>i</sup> isolates, all of which were ERY<sup>n</sup> or ERY<sup>i</sup>) were tested, and the MIC<sub>50</sub> and MIC<sub>90</sub> of ABT-773 were found to be 8 to 128 times and 16 to 32 times lower than the ERY MIC<sub>50</sub> and MIC<sub>90</sub> for both the VAN<sup>n</sup> and the VAN<sup>n</sup> or ERY<sup>n</sup> or ERY<sup>i</sup> groups, respectively. All *E. faecalis* isolates and 54 *E. faecium* isolates which had high-level ERY resistance (HLER) (ERY MIC, 256 to >512 μg/ml) tested positive for *erm*(B) (13). Of the 60 HLER isolates of *E. faecalis*, 20 were inhibited by ABT-773 at 0.015 to 0.5 μg/ml, 16 were inhibited by ABT-773 at 1 to 4 μg/ml, 13 were inhibited by ABT-773 at 8 to 32 μg/ml, and 11 were inhibited by ABT-773 at ≥64 μg/ml (Table 2). Similarly, of 54 HLER isolates of *E. faecium*, 4 were inhibited by ABT-773 at 0.25 μg/ml, 10 were inhibited by ABT-773 at 1 to 4 μg/ml, 37 were inhibited by ABT-773 at 8 to 32 μg/ml, and 3 were inhibited by ABT-773 at ≥64 μg/ml (Table 2). For all HLER *E. faecalis* isolates tested, CLF MICs were 32 to ≥256 μg/ml, while for 52 of 54 HLER *E. faecium* isolates CLF MICs were ≥256 μg/ml.

Of 29 group A streptococci and 10 group B streptococci tested, only one isolate, a group A streptococcus was ERY<sup>+</sup> (MIC, 16 μg/ml); the MIC of ABT-773 for that isolate was 0.062 μg/ml. ABT-773 was found to be more active against both group A and group B streptococcal strains, with MIC<sub>50</sub> and MIC<sub>90</sub> being four or more times lower than those of ERY, CLI, and Q-D. ABT-773 had MIC<sub>50</sub> 128, 4, and 64 times lower than those of ERY, CLI, and Q-D, respectively, for 21 alpha-hemolytic streptococci, which included 13 ERY<sup>n</sup> isolates and 8 ERY<sup>i</sup> isolates.

All 17 ERY<sup>n</sup> *S. pneumoniae* isolates were inhibited by ≤0.031 μg of ABT-773 per ml, while 90% of these isolates were inhibited by 0.125 μg of ERY and CLI per ml and 2 μg of Q-D per ml. Of 25 ERY<sup>a</sup> *S. pneumoniae* isolates tested, 5 isolates contained the *erm*(A) gene (13) and 18 contained the *erm*(B) gene (13). These 18 isolates with the *erm*(B) gene consisted of 9 isolates for which ERY MICs were 1 to >128 μg/ml and CLF MICs were 0.031 to 0.25 μg/ml and 9 isolates for which ERY MICs were <128 μg/ml and CLF MICs were >16 μg/ml. Twenty-four of the 25 ERY<sup>a</sup> *S. pneumoniae* isolates tested were inhibited by ABT-773 at ≤0.025 μg/ml, whereas the MIC<sub>50</sub> of ERY, CLI, and Q-D for these isolates were >128, >16, and 2 μg/ml, respectively; the remaining one *erm*(B) isolate was inhibited by ABT-773 at 2 μg/ml, which was several-fold higher than the MICs for other *erm*(B)-containing *S. pneumoniae* isolates.

Of 14 *Rhodococcus* spp. tested, 4 isolates were inhibited by 0.06 to 0.5 μg of ERY per ml and 10 were inhibited by 2 to 16 μg of ERY per ml; the MIC<sub>50</sub> of ABT-773 was 8 to 256 times lower than the ERY<sub>50</sub> of ERY, CLI, or Q-D for these isolates. *Leuconostoc* spp., *Pediococcus* spp., *Corynebacterium* spp., and *Lactobacillus* spp. were inhibited by ABT-773 at concentrations in the range of ≤0.007 to 0.062 μg/ml, while these isolates were inhibited by ERY at concentrations in the range of 0.062 to 16 μg/ml.

Broth microdilution MIC results for ABT-773 for all bacteria tested were either the same as or within 1 dilution of the agar dilution MICs (data not shown). The results of the present study show that ABT-773 is more potent than ERY against almost all isolates. However, the MICs of ABT-773 tended to vary with the MIC of ERY. The enterococcal isolates which were susceptible or intermediate in susceptibility to ERY were highly susceptible to ABT-773, while for isolates which were highly resistant to ERY, the MICs of ABT-773 were usually higher. The MIC<sub>50</sub> and MIC<sub>90</sub> of ABT-773 for ERY<sup>n</sup> isolates were 0.031 and 0.25 μg/ml, except one *erm*(B) isolate, suggesting a possibility that other mechanisms are respectively, for all involved in the higher MIC for this strain.

A macrolide efflux pump encoded by *erm*(A) and ribosomal alterations encoded by *erm*(B) have been described as the major mechanisms of ERY resistance in *S. pneumoniae* isolates in the literature (7, 14). In a study with MLS-resistant *S. pneumoniae* isolates, ABT-773 was shown to still have binding affinity for methylated ribosomes (J. O. Capobianco, V. Shortridge, Z. Ma, L. Phan, Y. S. Or, and P. Zhong, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2134, 1999). It has also been suggested that the effectiveness of ABT-773 in *mer*(A)-containing macrolide-resistant *S. pneu-
On the basis of the potent activity of ABT-773 against the Streptococcus spp. tested, and the lower MICs of ABT-773 for most of the Enterococcus spp. tested and the lower MICs of ABT-773 for Streptococcus (Capobianco et al., 39th ICAAC).

The capacity of the pump to remove the ketolide from the cell flux rate exceeded the flux pump or the fact that the influx rate exceeded the capacity of the pump to remove the ketolide from the cell (Capobianco et al., 39th ICAAC).

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We thank Audrey Wanger, University of Texas Health Science Center, and Kenneth V. I. Rolston, M. D. Anderson Cancer Center, Houston, Tex., for providing some of the isolates.

REFERENCES