In Vitro Bactericidal Activities of ABT-773 against ermB Strains of Streptococcus pneumoniae

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The bactericidal activities of ABT-773, a new ketolide, were compared to those of cefuroxime and amoxicillin-clavulanate against 10 strains of Streptococcus pneumoniae containing the ermB gene. MICs and time-kill curves were determined in duplicate per NCCLS guidelines with cation-adjusted Mueller-Hinton broth with 3% lysed horse blood. Viable counts were done at 0, 2, 6, and 24 h. Antibiotic concentrations tested were two and eight times the MIC. ABT-773 MICs ranged from 0.008 to 1.0 μg/ml. Bactericidal activity was observed with ABT-773 at eight times the MIC against 4 of 10 strains at 24 h compared to 10 of 10 strains with the beta-lactam antibiotics.

The ketolides, semisynthetic 14-membered ring macrolides, represent a new subclass of agents in the macrolide-lincosamide-streptogramin group. The substitution of the L-cladinose sugar with a 3-keto group on the erythronolide A ring is the major differing structural component of the ketolides (2). Despite structural similarity, the ketolides maintain in vitro activity against macrolide-resistant strains of Streptococcus pneumoniae. One of the newest agents, ABT-773, has demonstrated higher rates of accumulation as well as stronger ribosome binding than erythromycin with macrolide-resistant strains of S. pneumoniae (2). ABT-773 has also demonstrated the ability to accumulate in S. pneumoniae isolates containing the mef gene. Even in the presence of the erm gene, ABT-773 was shown to bind to methylated ribosomes, although binding was less than that to the wild type. It is these properties that allow the ketolide to remain active against macrolide-resistant organisms (2). The MIC of ABT-773 at which 90% of the isolates tested is inhibited has been reported previously to be 0.125 μg/ml for 78 ermB- and 44 mefE-containing strains (3).

With in vitro activity against macrolide-resistant S. pneumoniae (1), the ketolides are prime candidates for the treatment of community-acquired respiratory tract infections. The purpose of this study was to compare the in vitro bactericidal activities of ABT-773, amoxicillin-clavulanate, and cefuroxime against ermB-containing strains of S. pneumoniae.


MIC and time-kill assays were performed on 10 clinical isolates of S. pneumoniae containing the ermB gene. PCR was used to determine the genes present in the macrolide-resistant strains of S. pneumoniae. Bacterial DNA was prepared with the Wizard genomic DNA purification kit (Promega, Madison, Wis.). Primers (mefE and ermB) were prepared at the University of Illinois at Chicago Protein Research Laboratory as previously described (8, 9).

The isolates were stored at −70°C and underwent three subcultures prior to MIC and time-kill studies. The control strain, S. pneumoniae ATCC 49619, was used for validation of MIC results (5). For each experiment, the organisms were freshly subcultured on blood agar plates (Remel, Lenexa, Kans.) and incubated overnight at 35°C with 5% CO2. Each inoculum was prepared by direct suspension and adjusted with sterile saline until the turbidity matched a 0.5 McFarland standard with a spectrophotometer at 625 nm. Each suspension was further diluted in broth to obtain a final inoculum of approximately 5 × 10^7 CFU/ml. The exact inoculum size was determined via colony counts.

ABT-773 (Abbott Laboratories, Abbott Park, Ill.), amoxicillin-clavulanate, cefuroxime, erythromycin (U.S. Pharmacopeia, Rockville, Md.), and penicillin G (Sigma, St. Louis, Mo.) powders were prepared according to NCCLS guidelines or

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<tr>
<th>Strain</th>
<th>MIC (μg/ml) of drug (interpretation)</th>
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<tr>
<td></td>
<td>ABT-773</td>
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<tr>
<td>SP-300</td>
<td>0.008</td>
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<tr>
<td>SP-430</td>
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<tr>
<td>SP-627</td>
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<tr>
<td>SP-3418</td>
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<tr>
<td>SP-3428</td>
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<tr>
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<tr>
<td>SP-3464</td>
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<tr>
<td>SP-3480</td>
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a S, sensitive; R, resistant; I, intermediate.

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FIG. 1. Time-kill curves for 10 clinical strains of *S. pneumoniae* containing the *ermB* gene. Symbols: *, control; ▲, ABT-773, eight times the MIC; △, ABT-773, twice the MIC; ■, cefuroxime, eight times the MIC; □, cefuroxime, twice the MIC; ○, amoxicillin-clavulanate, eight times the MIC; ●, amoxicillin-clavulanate, twice the MIC; dotted line, lower limit of detection.
manufacturer’s recommendations (6). Cation-supplemented Mueller-Hinton broth with 3% lysed horse blood (Remel) was the medium used for the MIC and time-kill assays. MICs were determined in duplicate per NCCLS guidelines by the broth microdilution method (6). The microtiter plates were incubated at 35°C and read at 20 h. The MIC was defined as the lowest concentration at which there was no visible growth.

The bactericidal activities of ABT-773, amoxicillin-clavulanate, and cefuroxime were determined in duplicate by the time-kill method per NCCLS guidelines (7). Antimicrobial concentrations tested were two and eight times the MIC. Test tubes with broth media and known concentrations of the antibiotics were inoculated with the organisms. Control tubes were utilized that contained no antimicrobial agent. The final inoculum was confirmed at time zero; subsequent viable counts were performed at 2, 6, and 24 h. Sampling for colony counts was done by removing 0.1 ml of broth at the specified times. Each sample was serially diluted with sterile saline to produce 10-fold dilutions. Dilutions were utilized to increase the accuracy of viable counts and to minimize antibiotic carryover. The diluted samples were plated on blood agar plates with a spiral plater (WASP spiral plater; Microbiology International, Frederick, Md.). Colonies were counted after 24 h of incubation at 35°C and read at 20 h. The MIC was determined in duplicate per NCCLS guidelines by the broth microdilution method (6). The microtiter plates were incubated at 35°C and read at 20 h. The MIC was defined as the 3-log10 decrease in CFU per milliliter, while bacteriostatic activity was defined as a <3-log10 decrease in CFU per milliliter (7). The lower limit of detection was 1.3 log10 CFU/ml.

The MICs of each agent are listed in Table 1. All 10 strains of Staphylococcus pneumoniae were resistant to erythromycin; 7 of the 10 isolates displayed high-level erythromycin resistance (≥256 µg/ml). ABT-773 exhibited activity against all strains, including the isolates with high-level erythromycin resistance. Based upon the proposed breakpoints for ABT-773 against S. pneumoniae (≥0.5 µg/ml for sensitive, 1 µg/ml for intermediate, and ≥2 µg/ml for resistant strains) (G. Stone, A. Nilius, D. Henley, L. Almer, J. Beyer, and R. Flamm, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2164, 2000), nine isolates would be considered sensitive while one strain would be intermediate.

The results of the time-kill studies are shown in Fig. 1. All agents were bacteriostatic at 2 h, while the beta-lactams demonstrated enhanced activity at 6 h. At 24 h, ABT-773 at eight times the MIC demonstrated bactericidal activity against four strains. In comparison, both two and eight times the MIC of cefuroxime and amoxicillin-clavulanate were bactericidal against all 10 strains.

Davies et al. compared the in vitro bactericidal activities of ABT-773 with those of 10 other agents against 12 clinical isolates of S. pneumoniae, including three strains carrying the ermB gene (3). The MICs of ABT-773 ranged from 0.004 to 0.06 µg/ml for the 12 strains. At 24 h, ABT-773 at two to four times the MIC was bactericidal against the three strains with high-level erythromycin resistance. Ramer et al. also reported that ABT-773 exhibited bactericidal activity at four to eight times the MIC against two strains of S. pneumoniae containing the ermAM gene (N. Ramer, D. McDaniel, P. Johnson, D. Shortridge, and R. K. Flamm, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2137, 2000). In contrast to these studies, we found ABT-773 at eight times the MIC to be bactericidal against only 4 of 10 strains carrying the ermB gene. Bactericidal activity was not observed with any of the 10 strains when tested at an ABT-773 concentration of twice the MIC. Strain variability is most likely responsible for the differences observed in the studies, as only a small number of isolates were tested.

Davies et al. also compared ABT-773 to beta-lactam agents commonly used in the treatment of community-acquired infections (3). At 24 h, concentrations of four times the MICs of amoxicillin and ceftriaxone were bactericidal against the 12 strains. Similar results were seen in our study. At 24 h, bactericidal activity was observed at two and eight times the MICs of cefuroxime and amoxicillin-clavulanate against all 10 strains.

With worldwide surveillance studies reporting increasing rates of penicillin- and macrolide-resistant strains of S. pneumoniae (4), there is a need for new agents that have activity against S. pneumoniae. ABT-773, a new ketolide, exhibits in vitro activity against high-level macrolide-resistant S. pneumoniae (1). In our study, ABT-773 demonstrated bacteriostatic activity against most strains of ermB-containing pneumococci, while the beta-lactams were bactericidal against all strains. Further studies are needed to examine the potential role of this new ketolide in the treatment of community-acquired respiratory tract infection.

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REFERENCES