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Susceptibilities of *Haemophilus influenzae* and *Moraxella catarrhalis* to ABT-773 Compared to Their Susceptibilities to 11 Other Agents

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The activity of the ketolide ABT-773 against *Haemophilus* and *Moraxella* was compared to those of 11 other agents. Against 210 *Haemophilus influenzae* strains (39.0% β-lactamase positive), microbroth dilution tests showed that azithromycin and ABT-773 had the lowest MICs (0.5 to 4.0 and 1.0 to 8.0 μg/ml, respectively), followed by clarithromycin and roxithromycin (4.0 to >32.0 μg/ml). Of the β-lactams, ceftriaxone had the lowest MICs (≤0.004 to 0.016 μg/ml), followed by cefixime and cefpodoxime (0.008 to 0.125 and ≤0.125 to 0.25 μg/ml, respectively), amoxicillin-clavulanate (0.125 to 4.0 μg/ml), and cefuroxime (0.25 to 8.0 μg/ml). Amoxicillin was only active against β-lactamase-negative strains, and cefprozil had the highest MICs of all oral cephalosporins tested (0.5 to >32.0 μg/ml). Against 50 *Moraxella catarrhalis* strains, all of the compounds except amoxicillin and cefprozil were active. Time-kill studies against 10 *H. influenzae* strains showed that ABT-773, at two times the MIC, was bactericidal against 9 of 10 strains, with 99% killing of all strains at the MIC after 24 h; at 12 h, ABT-773 gave 90% killing of all strains at two times the MIC. At 3 and 6 h, killing by ABT-773 was slower, with 99.9% killing of four strains at two times the MIC after 6 h. Similar results were found for azithromycin, with slightly slower killing by erythromycin, clarithromycin, and roxithromycin, especially at earlier times. β-Lactams were bactericidal against 8 to 10 strains at two times the MIC after 24 h, with slower killing at earlier time periods. Most compounds gave good killing of five *M. catarrhalis* strains, with β-lactams killing more rapidly than other drugs. ABT-773 and azithromycin gave the longest postantibiotic effects (PAEs) of the ketolide-macrolide-azalide group tested (4.4 to >8.0 h), followed by clarithromycin, erythromycin, and roxithromycin. β-Lactam PAEs were similar and shorter than those of the ketolide-macrolide-azalide group for all strains tested.

Although development of an effective vaccine against *Haemophilus influenzae* type b has led to the disappearance of the organism in many parts of the world, its place has been taken by untypeable *H. influenzae* strains. These organisms (followed by *Streptococcus pneumoniae* and *Moraxella catarrhalis*) are now considered to be the leading cause of acute exacerbations of chronic bronchitis and an important cause, together with *S. pneumoniae* and *M. catarrhalis*, of acute otitis media, sinusitis, and community-acquired respiratory tract infections (1, 8, 10, 12, 14, 23).

Current recommendations by the National Committee for Clinical Laboratory Standards (NCCLS) for use of *Haemophilus* test medium (HTM) for *Haemophilus* susceptibility testing (13) have been complicated by difficulty in commercial manufacture of this medium and its short half-life when made in house. Reliable *Haemophilus* susceptibility testing with HTM requires the use of freshly made medium within 3 weeks of manufacture (11, 22).

ABT-773 is a new ketolide (2; A. M. Nilius, M. Bui, L. Almer, D. Hensey, J. Boor, Z. Ma, Y. S. Ar, and R. Flamm, Abstr. 9th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P-177, 1999; Z. Ma, R. F. Clark, and Y. Or, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2133, 1999; Z. Cao, R. Hammond, S. Pratt, A. Saiki, C. Lerner, and P. Zhong, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2135, 1999). Previous preliminary studies have shown that this compound has low MICs against respiratory pathogens, including *Haemophilus* and *Moraxella* (2; D. Shortridge, N. C. Ramer, J. Boor, Z. Ma, Y. Or, and R. K. Flamm, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2136, 1999). This study further examined activity of ABT-773 against *Haemophilus* and *Moraxella* by (i) using NCCLS microdilution MIC methodology to test the activity of ABT-773 compared to those of erythromycin, azithromycin, clarithromycin, roxithromycin, amoxicillin, amoxicillin-clavulanate, cefuroxime, cefixime, cefpodoxime, cefprozil, and ceftriaxone against 210 *H. influenzae* and 50 *M. catarrhalis* strains; (ii) testing the kill kinetics of the above-mentioned compounds against 10 *H. influenzae* and 5 *M. catarrhalis* strains; and (iii) testing the postantibiotic effects (PAEs) of the above-mentioned compounds against 5 *H. influenzae* strains.

MATERIALS AND METHODS

Bacteria and antimicrobials. Strains (210 *H. influenzae* and 50 *M. catarrhalis*) were isolated from clinical specimens within the past 2 years and stored at −70°C in double-strength skim milk (Difco Laboratories, Detroit, Mich.) prior to use. ABT-773 susceptibility powder was obtained from Abbott Laboratories, Chicago, Ill. Other drugs were obtained from their respective manufacturers.

MIC determination. Microdilution MIC tests were performed by the NCCLS microdilution method (13). *H. influenzae* strains were all untypeable organisms. Inocula were prepared from chocolate agar plates incubated for a full 24 h by the direct colony suspension method as follows. In a tube of Mueller-Hinton broth (Difco), an organism suspension was made to a density of a 0.5 McFarland standard (10^8 CFU/ml). The inoculum was diluted in sterile saline such that final

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TABLE 1. MICs of 210 *H. influenzae* and 50 *M. catarrhalis* strains

Drug	β-lactamase-positive *H. influenzae* (82)[a]			β-lactamase-negative *H. influenzae* (128)			All *H. influenzae* (210)			*M. catarrhalis* (50)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
ABT-773	1.0–8.0	4.0	4.0	1.0–8.0	4.0	4.0	1.0–8.0	4.0	4.0	0.125–0.5	0.125	0.25
Erythromycin	2.0–16.0	8.0	16.0	2.0–>16.0	8.0	16.0	2.0–>16.0	8.0	16.0	0.25–1.0	0.5	0.5
Azithromycin	1.0–4.0	2.0	4.0	0.5–4.0	2.0	4.0	0.5–4.0	2.0	4.0	0.06–0.25	0.125	0.125
Clarithromycin	4.0–16.0	16.0	16.0	4.0–>32.0	8.0	16.0	4.0–>32.0	8.0	16.0	0.25–0.5	≤0.25	≤0.25
Roxithromycin	4.0–32.0	16.0	32.0	8.0–>32.0	16.0	32.0	4.0–>32.0	16.0	32.0	0.5–2.0	1.0	1.0
Amoxicillin	4.0–>16.0	>16.0	>16.0	0.125–2.0	0.5	1.0	0.125–>16.0	1.0	>16.0	2.0–>16.0	8.0	>16.0
Amoxicillin-clavulanate	0.5–4.0	1.0	2.0	0.125–2.0	0.5	1.0	0.125–4.0	1.0	2.0	0.125–0.5	≤0.125	0.25
Cefuroxime	0.25–8.0	0.5	1.0	0.25–4.0	0.5	1.0	0.25–8.0	0.5	1.0	0.25–8.0	2.0	4.0
Cefixime	0.008–0.06	0.03	0.06	0.008–0.125	0.03	0.03	0.008–0.125	0.03	0.06	0.008–1.0	0.25	0.5
Cefpodoxime	≤0.125–0.25	≤0.125	≤0.125	≤0.125–0.25	≤0.125	≤0.125	≤0.125–0.25	≤0.125	≤0.125	0.25–4.0	1.0	2.0
Cefprozil	1.0–>32.0	4.0	16.0	0.5–16.0	2.0	4.0	0.5–>32.0	4.0	8.0	0.25–>32.0	4.0	8.0
Ceftriaxone	≤0.004–0.016	≤0.004	0.008	≤0.004–0.016	≤0.004	≤0.004	≤0.004–0.016	≤0.004	≤0.004	0.016–2.0	0.5	2.0

[a] Number of strains.

organism suspensions in trays yielded colony counts of 3×10^5 to 8×10^5 CFU/ml (11).

Frozen microdilution trays were obtained from MicroMedia Systems, Inc. (Cleveland, Ohio). Each tray contained all antimicrobials prepared in freshly made HTM. The wells were inoculated with 5×10^5 CFU/ml and incubated in ambient air at 35°C for 20 to 24 h. The lowest drug concentration showing no growth was read as the MIC. Clavulanate was added to amoxicillin at a ratio of 1 to 2. Standard quality control strains, including *H. influenzae* ATCC 49766, *H. influenzae* ATCC 49247, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922 were included with each run.

Time-kill studies. Glass tubes containing 5 ml of HTM (freshly made, as described above) with doubling antibiotic concentrations were inoculated with approximately 5×10^5 CFU (5×10^5 to 5×10^6 CFU) of organism/ml and incubated at 35°C in a shaking water bath. Antibiotic concentrations were chosen to comprise 3 doubling dilutions above and 3 dilutions below the MIC. Freshly made batches of HTM were used for all tests. The dilutions required to obtain the correct inoculum (approximately 5×10^5 CFU/ml) were determined by prior viability studies using each strain (17–20).

To inoculate each tube of serially diluted antibiotic, 50 μl of diluted inoculum was delivered by pipette beneath the surface of the broth and then vortexed and plated for viability counts (zero hour). Only tubes containing an initial inoculum within the range of 5×10^5 to 5×10^6 CFU/ml were acceptable (17–20).

Viability counts of antibiotic-containing suspensions were performed at 0, 3, 6, 12, and 24 h by plating 10-fold dilutions of 0.1-ml aliquots from each tube in sterile HTM onto chocolate agar plates. Recovery plates were incubated for up to 48 h. Colony counts were performed on plates yielding 30 to 300 colonies (17–20).

The lower limit of sensitivity of colony counts was 300 CFU/ml.

Time-kills were analyzed by determining the number of strains which yielded a $\Delta\log_{10}$ CFU per milliliter of −1, −2, and −3 at 0, 3, 6, 12, and 24 h compared to counts at 0 h. Antimicrobials were considered bactericidal at the lowest concentration that reduced the original inoculum by ≥3 $\log_{10}$ CFU/ml (99.9%) at each of the time points and were considered bacteriostatic if the inoculum was reduced by 0 to 3 $\log_{10}$ CFU/ml. With the sensitivity threshold and inocula used in these studies, no problems were encountered in delineating 99.9% killing, when present. The problem of bacterial carryover was addressed as described previously (17–20).

Measurement of PAE. PAE was determined by the viable plate count method (4) using freshly made HTM (7, 11). The bacterial inoculum was prepared by suspending growth from an overnight chocolate agar plate in broth. The broth was incubated at 35°C for 2 to 4 h in a shaking water bath until the turbidity matched a no. 1 MacFarland standard (approximately 5×10^8 CFU/ml).

For PAE experiments, 5-ml tubes of broth containing the antibiotic concentrations to be tested at 2 times the MIC (cefprozil), 4 times the MIC (ABT-773, erythromycin, azithromycin, clarithromycin, roxithromycin, amoxicillin, and amoxicillin-clavulanate), and 10 times the MIC (cefuroxime, cefixime, cefpodoxime, and ceftriaxone) (concentrations are based upon pharmacokinetics) were inoculated with 50 μl of inoculum to provide 5×10^6 CFU/ml. The tubes were then vortexed and plated for viability counts. Growth controls with inoculum but no antibiotic were included with each experiment. The inoculated test tubes were then placed in a shaking water bath at 35°C for an exposure period of 1 h. At the end of the exposure period, the cultures were diluted 1:1,000 in prewarmed broth to remove the antibiotic. An additional control culture containing bacteria and antibiotic at a concentration of 0.01 times the MIC was prepared to confirm that after dilution the antibiotic was no longer bacteriostatic (4, 7).

Viability counts were determined before exposure and immediately after dilution (zero hour) and then every 2 h until the turbidity of the tube reached a no. 1 MacFarland standard. Viability counts were performed by preparing 10-fold dilutions of 0.1-ml aliquots from each tube in HTM and plating 0.1-ml volumes onto chocolate agar plates. Recovery plates were inoculated for at least 72 h, and colony counts were performed on plates yielding 30 to 300 colonies.

The PAE was defined according to Craig and Gudmundsson (4) as PAE = $T - C$, where T is the time required for viability counts of an antibiotic-exposed culture to increase by 1 $\log_{10}$ unit above the counts observed immediately after dilution and C is the corresponding time for the growth control.

For each experiment, viability counts, expressed as $\log_{10}$ CFU per milliliter, were plotted against time. The results were expressed as the mean of two separate assays.

RESULTS

The results of MIC testing of *H. influenzae* are presented in Table 1. Against 210 *H. influenzae* strains (39.0% β-lactamase positive), microbroth dilution tests showed that azithromycin and ABT-773 had the lowest MICs (0.5 to 4.0 and 1.0 to 8.0 μg/ml, respectively), followed by clarithromycin and roxithromycin (4.0 to >32.0 μg/ml). Of the β-lactams, ceftriaxone had the lowest MICs (≤0.004 to 0.016 μg/ml), followed by cefixime and cefpodoxime (0.008 to 0.125 and ≤0.125 to 0.25 μg/ml, respectively), amoxicillin-clavulanate (0.125 to 4.0 μg/ml) and cefuroxime (0.25 to 8.0 μg/ml). Amoxicillin was only active against β-lactamase-negative strains, and cefprozil had the highest MICs of all oral cephalosporins tested (0.5 to >32.0 μg/ml). Against 50 *M. catarrhalis* strains, all compounds except amoxicillin and cefprozil were active (Table 1).

The MICs of the 15 strains tested by time-kill were similar to those listed in Table 1. Kill kinetics results of the 10 *H. influenzae* strains are shown in Table 2, and those of the 5 *M. catarrhalis* strains are shown in Table 3. Two *H. influenzae* and all five *M. catarrhalis* strains were β-lactamase positive and were not tested by time-kill with amoxicillin. As can be seen, ABT-773, at two times the MIC, was bactericidal (99.9% killing) against 9 of 10 strains, with 99% killing of all strains at the MIC after 24 h; at 12 h, ABT-773 gave 90% killing of all strains at two times the MIC. At 3 and 6 h, killing by ABT-773 was slower, with 99.9% killing of four strains at two times the MIC after 6 h. Similar results were found for azithromycin, with

TABLE 2. Results of kill kinetics studies for 10 *H. influenzae* strains

Drug and concn	No. of strains											
	3 h			6 h			12 h			24 h		
	−1[a]	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
ABT-773												
4 × MIC	7	5	2	8	5	5	10	8	5	10	10	10
2 × MIC	6	2	1	8	5	4	10	7	5	10	10	9
MIC	2	1	0	4	2	0	8	4	4	10	10	6
0.5 × MIC	0	0	0	0	0	0	0	0	0	0	0	0
Erythromycin												
4 × MIC	5	2	1	7	5	2	10	6	5	10	10	9
2 × MIC	5	1	1	6	4	1	10	6	4	10	10	8
MIC	2	0	0	4	0	0	8	2	1	9	8	5
0.5 × MIC	0	0	0	0	0	0	1	0	0	0	0	0
Azithromycin												
4 × MIC	7	4	3	10	6	5	10	10	9	10	10	10
2 × MIC	4	3	1	9	5	3	10	10	7	10	10	10
MIC	3	1	0	6	2	0	9	4	3	9	9	7
0.5 × MIC	0	0	0	0	0	0	1	1	0	1	1	0
Clarithromycin												
4 × MIC	5	2	0	8	5	2	10	6	5	10	10	9
2 × MIC	4	1	0	7	4	2	10	5	4	10	10	9
MIC	1	0	0	2	0	0	8	2	0	7	7	3
0.5 × MIC	0	0	0	0	0	0	0	0	0	0	0	0
Roxithromycin												
4 × MIC	5	3	1	8	5	2	10	6	5	10	10	9
2 × MIC	4	2	1	7	4	2	9	6	4	10	10	9
MIC	2	0	0	3	1	0	6	4	3	7	7	3
0.5 × MIC	0	0	0	0	0	0	0	0	0	0	0	0
Amoxicillin												
4 × MIC	6[b]	0	0	8	2	0	8	7	3	8	8	8
2 × MIC	4	0	0	7	0	0	8	5	1	8	8	7
MIC	3	0	0	5	0	0	5	3	0	6	4	3
0.5 × MIC	0	0	0	0	0	0	1	0	0	1	0	0
Amoxicillin-clavulanate												
4 × MIC	4	1	0	9	3	0	10	9	3	10	10	9
2 × MIC	3	0	0	9	3	0	10	9	3	10	10	9
MIC	3	0	0	7	2	0	10	4	2	10	7	5
0.5 × MIC	0	0	0	0	0	0	1	0	0	1	0	0
Cefuroxime												
4 × MIC	5	0	0	10	2	0	10	9	4	10	10	10
2 × MIC	4	0	0	9	2	0	10	8	3	10	10	8
MIC	2	0	0	5	0	0	8	4	1	8	8	6
0.5 × MIC	0	0	0	1	0	0	2	0	0	1	0	0
Cefixime												
4 × MIC	4	1	0	8	5	1	9	7	5	10	10	10
2 × MIC	4	0	0	8	5	1	10	8	3	10	10	9
MIC	3	0	0	8	4	0	10	8	3	10	10	8
0.5 × MIC	0	0	0	1	0	0	2	1	0	2	0	0
Cefpodoxime												
4 × MIC	5	1	0	8	4	0	10	7	2	10	10	9
2 × MIC	3	0	0	8	5	0	10	8	4	10	10	9
MIC	3	0	0	7	4	0	9	6	3	10	9	7
0.5 × MIC	1	0	0	1	0	0	3	0	0	0	0	0
Cefprozil												
4 × MIC	5	0	0	10	6	0	10	10	6	10	10	10
2 × MIC	4	0	0	10	4	0	10	8	5	10	10	10
MIC	1	0	0	5	2	0	9	3	2	9	8	6
0.5 × MIC	0	0	0	2	0	0	1	0	0	0	0	0
Ceftriaxone												
4 × MIC	3	0	0	8	6	2	10	8	6	10	10	9
2 × MIC	3	0	0	8	5	1	10	8	6	10	10	9
MIC	2	0	0	8	3	0	10	8	3	10	9	8
0.5 × MIC	0	0	0	0	0	0	3	0	0	0	0	0

[a] ΔLog_{10} CFU per milliliter lower than at 0 h.
[b] Only tested against β-lactamase-negative strains.

TABLE 3. Results of kill kinetics studies for five *M. catarrhalis* strains[a]

Drug and concn	No. of strains											
	3 h			6 h			12 h			24 h		
	−1[b]	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
ABT-773												
4 × MIC	0	0	0	0	0	0	4	3	0	5	5	5
2 × MIC	0	0	0	0	0	0	4	1	0	5	5	4
MIC	0	0	0	0	0	0	1	0	0	5	5	3
0.5 × MIC	0	0	0	0	0	0	0	0	0	1	1	1
Erythromycin												
4 × MIC	0	0	0	1	0	0	5	3	1	5	5	5
2 × MIC	0	0	0	0	0	0	4	2	0	5	5	5
MIC	0	0	0	0	0	0	4	0	0	5	5	5
0.5 × MIC	0	0	0	0	0	0	0	0	0	2	0	0
Azithromycin												
4 × MIC	0	0	0	4	0	0	5	5	3	5	5	5
2 × MIC	0	0	0	1	0	0	5	5	2	5	5	5
MIC	0	0	0	0	0	0	5	0	0	5	5	5
0.5 × MIC	0	0	0	0	0	0	0	0	0	1	0	0
Clarithromycin												
4 × MIC	0	0	0	1	0	0	5	2	1	5	5	4
2 × MIC	0	0	0	0	0	0	4	2	0	5	5	4
MIC	0	0	0	0	0	0	2	0	0	5	5	3
0.5 × MIC	0	0	0	0	0	0	0	0	0	1	1	0
Roxithromycin												
4 × MIC	0	0	0	2	0	0	4	3	0	5	5	5
2 × MIC	0	0	0	0	0	0	4	1	0	5	5	5
MIC	0	0	0	0	0	0	2	0	0	5	4	3
0.5 × MIC	0	0	0	0	0	0	0	0	0	0	0	0
Amoxicillin-clavulanate												
4 × MIC	4	1	0	5	5	1	5	5	5	5	5	5
2 × MIC	4	1	0	5	5	1	5	4	3	5	5	5
MIC	3	0	0	5	3	0	5	3	1	5	4	1
0.5 × MIC	1	0	0	4	1	0	1	1	1	1	0	0
Cefuroxime												
4 × MIC	5	3	0	5	5	3	5	5	3	5	5	5
2 × MIC	5	3	0	5	5	3	5	4	2	5	5	4
MIC	5	1	0	5	4	1	5	2	1	3	0	0
0.5 × MIC	1	1	0	3	1	0	3	0	0	0	0	0
Cefixime												
4 × MIC	3	2	0	4	3	2	5	5	3	5	5	5
2 × MIC	3	0	0	4	3	1	5	5	2	5	4	2
MIC	2	0	0	3	2	0	4	2	0	3	2	0
0.5 × MIC	0	0	0	1	0	0	2	0	0	0	0	0
Cefpodoxime												
4 × MIC	5	5	0	5	5	4	5	5	5	5	5	4
2 × MIC	5	1	0	5	5	2	5	5	3	5	3	2
MIC	3	0	0	5	3	1	5	3	1	3	2	1
0.5 × MIC	1	0	0	3	1	1	2	1	0	0	0	0
Cefprozil												
4 × MIC	5	4	1	5	5	3	5	5	3	5	4	2
2 × MIC	5	4	0	5	5	3	5	4	2	2	0	0
MIC	5	3	0	5	5	3	5	4	0	0	0	0
0.5 × MIC	5	2	0	4	3	2	4	1	0	0	0	0
Ceftriaxone												
4 × MIC	5	1	0	5	5	1	5	5	3	5	4	1
2 × MIC	4	0	0	5	4	0	5	5	2	5	2	0
MIC	4	0	0	5	1	0	5	4	1	2	0	0
0.5 × MIC	0	0	0	4	0	0	4	0	0	0	0	0

[a] Amoxicillin was not tested (all strains are β-lactamase positive).
[b] ΔLog_{10} CFU per milliliter lower than at 0 h.

TABLE 4. MICs and PAEs of five *H. influenzae* strains

Drug	MIC range (μg/ml)	PAE (h)[a]
ABT-773[b]	1.0–8.0	≥6.1 (4.9–>8.0)
Erythromycin[b]	4.0–16.0	≥3.8 (0.9–>6.7)
Azithromycin[b]	2.0–8.0	≥6.1 (4.4–>7.4)
Clarithromycin[b]	4.0–16.0	≥3.3 (1.6–>6.7)
Roxithromycin[b]	8.0–32.0	≥3.3 (1.9–>6.7)
Amoxicillin[b]	0.5–4.0	0.5 (0–2.6)
Amoxicillin-clavulanate[b]	0.5–1.0	0.7 (0–3.4)
Cefuroxime[c]	0.5–1.0	1.0 (0–5.0)
Cefixime[c]	0.016–0.125	0.7 (0–3.7)
Cefpodoxime[c]	0.125–0.25	0.7 (0–3.5)
Cefprozil[d]	4.0–16.0	0.7 (0–3.3)
Ceftriaxone[c]	0.004–0.008	0.6 (0–3.2)

[a] Mean (range).
[b] Exposure at 4 times MIC for 1 h.
[c] Exposure at 10 times MIC for 1 h.
[d] Exposure at 2 times MIC for 1 h.

slightly slower killing by erythromycin, clarithromycin, and roxithromycin, especially at earlier time periods. β-Lactams were bactericidal against 8 to 10 strains at two times the MIC after 24 h, with slower killing at earlier time periods.

Time-kill studies for the five *M. catarrhalis* strains (Table 3) showed that all compounds except cefprozil and ceftriaxone were bactericidal at or above the MIC after 24 h, with other β-lactams showing more rapid killing at earlier time periods.

PAEs are presented in Table 4. As can be seen, ABT-773 and azithromycin gave the longest PAEs of the ketolide-macrolide-azalide group tested (4.4 to >8.0 h), followed by clarithromycin, erythromycin, and roxithromycin. β-Lactam PAEs were all similar and shorter than those of the ketolide-macrolide-azalide group for all strains tested.

DISCUSSION

ABT-773 is a new ketolide (Ma et al., Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., 1999; Cao et al., Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., 1999) which, in preliminary studies, has been reported to be more potent in vitro than the macrolides against *H. influenzae*, *M. catarrhalis*, *Legionella* spp., *Neisseria gonorrhoeae*, and *Listeria monocytogenes*. ABT-773 was also more potent against macrolide-susceptible strains of *S. pneumoniae*, *Streptococcus pyogenes*, *S. aureus*, *Staphylococcus epidermidis*, enterococci, *Helicobacter pylori*, and *Mycobacterium avium* complex and also against *Corynebacterium* spp., *Mycoplasma pneumoniae*, *Chlamydia trachomatis*, *Borrelia burgdorferi*, and *Toxoplasma gondii*. ABT-773 had potent activity against macrolide-resistant streptococci and enterococci irrespective of their macrolide resistance mechanisms but had little detectable activity against constitutively macrolide-resistant staphylococci and macrolide-resistant *H. pylori* and *M. avium* complex (2; Shortridge et al., Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., 1999; M. M. Neuhauser, J. L. Prause, R. Jung, N. Boyea, J. M. Hackleman, L. H. Danziger, and S. L. Pendland, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr 2139, 1999; F. Goldstein, M. D. Kitzis, M. Miegi, and J. F. Acar, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2142, 1999; A. L. Barry, P. C. Fuchs, and S. D. Brown, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2144, 1999; S. L. Pendland, J. L. Prause, M. M. Neuhauser, N. Boyea, J. M. Hackleman, and L. H. Danziger, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2145, 1999; R. Jung, D. H. Li, S. L. Pendland, and L. H. Danziger, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2146, 1999; A. A. Khan, F. G. Araujo, J. C. Craft, and J. S. Remington, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2147, 1999). ABT-773 has been shown to be effective against *S. pneumoniae* in a rat lung model (J. Meulbroek, M. Mitten, K. W. Mollison, P. Ewing, J. Alder, A. M. Nilius, R. K. Flamm, Z. Ma, and Y. Or, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2151, 1999).

In our study, the MICs of ABT-773 against *H. influenzae* and *M. catarrhalis* were similar to those recently reported by others (2; J. Dubois, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2163, 2000; T. Fujikawa, S. Miyazaki, T. Matsumoto, A. Ohno, N. Furuya, Y. Ishii, K. Tateda, and K. Yamaguchi, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2166, 2000), with MICs at which 90% of the strains are inhibited (MIC_{90}s) of 4.0 μg/ml against *H. influenzae* and 0.06 to 0.25 μg/ml for *M. catarrhalis*. A recent study (V. Shortridge, N. Ramer, D. McDaniel, P. Johnson, and R. K. Flamm, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2137, 2000) has documented that, similar to our findings, ABT-773 was bactericidal against *H. influenzae* at four and eight times the MIC, with more rapid killing than erythromycin. ABT-773 was also found to have a longer PAE than erythromycin.

Previous studies have shown, similar to the findings reported here, that azithromycin was the most potent member of the macrolide-azalide-ketolide group by MIC and time-kill against *H. influenzae* strains, followed by the ketolides telithromycin, clarithromycin, and roxithromycin (9, 15–17, 21). In the present study, ABT-773 had kill kinetics against *H. influenzae* and *M. catarrhalis* comparable to that of azithromycin, the macrolide with the greatest overall in vitro activity against this group (9, 17), and also had the longest PAE of all compounds tested.

The clinical application of macrolide MICs against *H. influenzae* is a complex problem. Macrolides, azalides, and ketolides all exhibit a unimodal MIC distribution against this species, and macrolide resistance mechanisms similar to ribosomal methylase and efflux in gram-positive species have not been clearly defined. Also, there is a question concerning the validity of established breakpoints for macrolides and azalides against *H. influenzae*. Craig (3) has suggested that breakpoints for azithromycin and clarithromycin against *Haemophilus* are considerably lower than currently approved values in light of pharmacokinetic and pharmacodynamic parameters (11) and bacteriological outcome studies in otitis media (5, 6). Andes and Craig (Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2139, 2000) have demonstrated that ABT-773, like azithromycin and telithromycin, exhibits pharmacodynamic properties which correlate best with the area under the concentration-time curve/MIC ratio for *S. aureus* and *S. pneumoniae*. More detailed data on the free area under the concentration-time curve and MIC, as well as clinical studies, will be necessary to test the clinical validity of the above in vitro data. Long PAEs support once-daily dosing with ABT-773, similar to macrolides and azalides.

A tentative ABT-773 susceptibility breakpoint of ≤4.0 μg/ml

against *H. influenzae* has been proposed (G. Stone, A. Nilius, D. Hensey-Rudloff, L. Almer, J. Beyer, and R. Flamm, abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2164, 2000). Other factors, such as the increased concentration of compounds like clarithromycin in epithelial lining fluid (11) and the known anti-inflammatory effect of this group of agents may also play a role. Whether this phenomenon plays a role with ABT-773 remains to be established. Because of their low MICs, good kill kinetics, and long PAEs, azithromycin and ABT-773 appear to be the most potent agents of this group against *H. influenzae* on the basis of in vitro results.

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REFERENCES

1. **Berk, S. L., J. H. Kalbfleisch, and the Alexander Project Collaborative Group.** 1996. Antibiotic susceptibility patterns of community-acquired respiratory isolates of *Moraxella catarrhalis* in Western Europe and in the USA. J. Antimicrob. Chemother. **38**(Suppl. A)**:**85–96.
2. **Brueggemann, A. B., G. V. Doern, H. K. Huynh, E. M. Wingert, and P. R. Rhomberg.** 2000. In vitro activity of ABT-773, a new ketolide, against recent clinical isolates of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. Antimicrob. Agents Chemother. **44:**447–449.
3. **Craig, W. A.** 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin. Infect. Dis. **26:**1–12.
4. **Craig, W. A., and S. Gudmundsson.** 1996. Postantibiotic effect, p. 265–329. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams and Wilkins Co., Baltimore, Md.
5. **Dagan, R., C. E. Johnson, S. McLinn, N. Abughali, J. Feris, E. Leibovitz, D. J. Burch, and M. R. Jacobs.** 2000. Bacteriologic and clinical efficacy of amoxicillin/clavulanate vs. azithromycin in acute otitis media. Pediatr. Infect. Dis. J. **19:**95–104.
6. **Dagan, R., E. Leibovitz, D. M. Fliss, A. Lieberman, M. R. Jacobs, W. Craig, and P. Yagupsky.** 2000. Bacteriologic efficacies of oral azithromycin and oral cefaclor in treatment of acute otitis media in infants and young children. Antimicrob. Agents Chemother. **44:**43–50.
7. **Davies, T. A., L. M. Kelly, D. B. Hoellman, L. M. Ednie, C. L. Clark, S. Bajaksouzian, M. R. Jacobs, and P. C. Appelbaum.** 2000. Activities and postantibiotic effects of gemifloxacin compared to those of 11 other agents against *Haemophilus influenzae* and *Moraxella catarrhalis*. Antimicrob. Agents Chemother. **44:**633–639.
8. **Fang, G. D., M. Fine, J. Orloff, D. Arisumi, V. L. Yu, W. Kapoor, J. T. Grayston, S. P. Wang, R. Kohler, R. R. Muder, Y. C. Yee, J. D. Rihs, and R. M. Vickers.** 1990. New and emerging etiologies for community-acquired pneumonia with implications for therapy. A prospective multicenter study of 359 cases. Medicine (Baltimore) **69:**307–316.
9. **Goldstein, F. W., M. E. Emiran, A. Coutrot, and J. F. Acar.** 1990. Bacteriostatic and bactericidal activity of azithromycin against *Haemophilus influenzae*. J. Antimicrob. Chemother. **25**(Suppl. A)**:**25–28.
10. **Hoberman, A., J. L. Paradise, S. Block, D. J. Burch, M. R. Jacobs, and M. I. Balanescu.** 1996. Efficacy of amoxicillin/clavulanate for acute otitis media: relation to *Streptococcus pneumoniae*. Pediatr. Infect. Dis. **10:**955–962.
11. **Jacobs, M. R., S. Bajaksouzian, A. Zilles, G. Lin, G. A. Pankuch, and P. C. Appelbaum.** 1999. Susceptibilities of *Streptococcus pneumoniae* and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 U.S. surveillance study. Antimicrob. Agents Chemother. **43:**1901–1908.
12. **Maskell, J. P., A. M. Sefton, and J. D. Williams.** 1990. Comparative in-vitro activity of azithromycin and erythromycin against gram-positive cocci, *Haemophilus influenzae* and anaerobes. J. Antimicrob. Chemother. **25**(Suppl. A)**:** 19–24.
13. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS publication no. M7–A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
14. **Needham, C. A.** 1988. *Haemophilus influenzae*: antibiotic susceptibility. Clin. Microbiol. Rev. **1:**218–227.
15. **Neu, H. C.** 1991. The development of macrolides: clarithromycin in perspective. J. Antimicrob. Chemother. **27**(Suppl. A)**:**1–9.
16. **Olsson-Liljequist, B., and B.-M. Hoffman.** 1991. In-vitro activity of clarithromycin combined with its 14-hydroxy metabolite against *Haemophilus influenzae*. J. Antimicrob. Chemother. **27**(Suppl. A)**:**11–17.
17. **Pankuch, G. A., D. B. Hoellman, G. Lin, S. Bajaksouzian, M. R. Jacobs, and P. C. Appelbaum.** 1998. Activity of HMR 3647 compared to those of five agents against *Haemophilus influenzae* and *Moraxella catarrhalis* by MIC determination and time-kill assay. Antimicrob. Agents Chemother. **42:**3032–3034.
18. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1994. Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin and vancomycin by using time-kill methodology. Antimicrob. Agents Chemother. **38:**2065–2072.
19. **Pankuch, G. A., C. Lichtenberger, M. R. Jacobs, and P. C. Appelbaum.** 1996. Antipneumococcal activities of RP 59500 (quinupristin/dalfopristin), penicillin G, erythromycin, and sparfloxacin determined by MIC and rapid time-kill methodologies. Antimicrob. Agents Chemother. **40:**1653–1656.
20. **Pankuch, G. A., M. A. Visalli, M. R. Jacobs, and P. C. Appelbaum.** 1998. Susceptibilities of penicillin- and erythromycin-susceptible and -resistant pneumococci to HMR 3647 (RU 66647), a new ketolide, compared with susceptibilities to 17 other agents. Antimicrob. Agents Chemother. **42:**624–630.
21. **Vallée, E., A. Azoulay-Dupuis, R. Swanson, E. Bergogne-Bérézin, and J.-J. Pocidalo.** 1991. Individual and combined activities of clarithromycin and its 14-hydroxy metabolite in a murine model of *Haemophilus influenzae* infection. J. Antimicrob. Chemother. **27**(Suppl. A)**:**31–41.
22. **Yeo, S. F., E. Akalin, S. Arikan, R. Auckenthaler, T. Bergan, K. Dornbusch, A. J. Howard, W. Hryniewicz, R. N. Jones, G. Koupari, N. J. Legakis, J. McLaughlin, C. Ozkuyumcu, A. Percival, I. Phillips, D. Reeves, R. Spencer, R. E. Warren, and J. D. Williams.** 1996. Susceptibility testing of *Haemophilus influenzae*—an international collaborative study in quality assessment. J. Antimicrob. Chemother. **38:**363–386.
23. **Zeckel, M. L., K. D. Jacobson, F. J. Guerra, D. G. Therasse, and D. Farlow.** 1992. Loracarbef (LY163892) versus amoxicillin/clavulanate in the treatment of acute bacterial exacerbations of chronic bronchitis. Clin. Ther. **14:**214–229.