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Comparative In Vitro Activity of ABT-773, a Novel Antibacterial Ketolide

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The in vitro activities of ABT-773, erythromycin, clarithromycin, and azithromycin were compared. ABT-773 was the most active compound against macrolide-susceptible *Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes*, and *Enterococcus* spp. and multidrug-resistant *Streptococcus pneumoniae*. It also had good activity against gram-negative and atypical respiratory tract pathogens and *Helicobacter pylori*.

Macrolide antibiotics are used for treating community-acquired respiratory tract infections (1). Ketolides, erythromycin analogs in which a ketone functionality replaces the three-position cladinose, demonstrate in vitro antibacterial activity and in vivo efficacy in animal models of infection (3, 11). In addition, many ketolides retain potency against macrolide-resistant strains of streptococci (2, 3, 11) due to dimethylation of the 23S rRNA by Erm methylases or by macrolide-specific efflux pumps (3, 14, 15).

In addition to the ketone group at position 3, the novel ketolide ABT-773 is modified by an *O*-allyl-3-quinoline at the 6 position and a cyclized carbamate group between the 11 and 12 positions (11). This study evaluated the potency and spectrum of ABT-773. The MIC ranges and MICs inhibiting 50 (MIC_{50}) and 90% (MIC_{90}) of the tested strains are presented in Tables 1 and 2.

ABT-773, azithromycin, and clarithromycin were prepared at Abbott Laboratories, Abbott Park, Ill. Erythromycin, clindamycin, and penicillin reference powders were purchased from U.S. Pharmacopeial Convention, Inc., Rockville, Md.

Clinical isolates or reference strains obtained from the American Type Culture Collection (Manassas, Va.) were tested. The molecular mechanisms of macrolide resistance were identified by PCR amplification of the *mef* and *erm* genes (14, 15) and in *Helicobacter pylori* and *Mycobacterium avium* by DNA sequence analysis for mutations in 23S rRNA (8, 16).

MICs were determined by agar dilution or broth microdilution as described by the National Committee for Clinical Laboratory Standards (NCCLS) (9). Mueller-Hinton agar was supplemented with 5% sheep blood for testing *Streptococcus pyogenes, Listeria monocytogenes,* and *Moraxella catarrhalis;* the plates were incubated in an atmosphere containing 5% CO_2 for tests of *S. pyogenes* and *M. catarrhalis*. Quality control results met NCCLS standards (9, 10). The susceptibilities of *Legionella* spp., *M. avium, Mycoplasma pneumoniae, Borrelia burgdorferi,* and *Chlamydia trachomatis* were determined as described previously (5, 6, 7, 13, 18).

ABT-773 was at least fourfold more potent than the three comparator macrolides against macrolide-susceptible strains of gram-positive species, including *Streptococcus pneumoniae, S. pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, L. monocytogenes,* and *Enterococcus* spp; the strains were inhibited by <0.06 μg of ABT-773/ml. ABT-773 was as potent as azithromycin against the gram-negative pathogens *Haemophilus influenzae, M. catarrhalis, Legionella* spp., and *Neisseria gonorrhoeae* with MIC_{90}s of 4, 0.12, 1, and 0.25 μg/ml, respectively. ABT-773 had little to no activity against *Escherichia coli,* other enterobacteriaceae, and *Pseudomonas aeruginosa* (MICs, ≥8 μg/ml) [data not shown]. ABT-773 was highly active against *M. pneumoniae,* demonstrating MICs of <0.015 μg/ml. The MIC for ABT-773 against a single strain of *C. trachomatis* was 0.015 μg/ml, similar to the activity against *Chlamydia pneumoniae* (17).

The MIC_{90}s for ABT-773 were 0.25 and 0.03 μg/ml for inducible ErmA and susceptible strains of *S. aureus* and *S. pyogenes*. In contrast, macrolide MICs were more than 100-fold higher against inducible ErmA strains than against susceptible strains. Moreover, ABT-773 did not induce resistance to clindamycin in these strains by the disk approximation test; erythromycin induced resistance to both ABT-773 and clindamycin (data not shown; 12). These results suggest that ABT-773 does not induce 23S rRNA methylation in inducible ErmA strains.

Although the macrolides and clindamycin were inactive against constitutive ErmB strains of *S. pneumoniae* and *S. pyogenes,* the ABT-773 MIC_{90} for ErmB strains of *S. pneumoniae* was 0.25 μg/ml, and all ErmB strains of *S. pyogenes* were inhibited by ≤8 μg/ml. The difference in activity of ABT-773 was likely due to the use of agar dilution with incubation in CO_2 to test *S. pyogenes* and broth microdilution to test *S. pneumoniae* and not to inherent species-dependent differences in susceptibility to ABT-773, since other studies show that the MIC_{90}s for ErmB strains of *S. pneumoniae* and *S. pyogenes* are similar when the susceptibilities of both species are determined by broth microdilution (V. D. Shortridge, unpublished data). The potent activity of ABT-773 against constitutive ErmB streptococci may be due to its affinity for methylated ribosomes, and ABT-773 may not effectively induce higher levels of ribosome methylation in ErmB strains (4). This may also ex-

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TABLE 1. Comparative in vitro activity of ABT-773 by agar dilution methods

Species (resistance) [*n*] and antibiotic	MIC (μg/ml)		
	Range	50%	90%
S. pyogenes (macrolide S) [17]			
ABT-773	≤0.015–0.03	0.03	0.03
Clarithromycin	0.03	0.03	0.03
Azithromycin	0.12–0.25	0.12	0.25
Erythromycin	0.03–0.06	0.03	0.06
Clindamycin	0.03–0.06	0.03	0.06
S. pyogenes (MefA) [13]			
ABT-773	≤0.015–0.25	0.12	0.25
Clarithromycin	2–16	4	16
Azithromycin	2–8	8	8
Erythromycin	2–16	8	16
Clindamycin	≤0.015–0.06	0.06	0.06
S. pyogenes (inducible ErmA) [11]			
ABT-773	≤0.015–>1	0.03	0.12
Clarithromycin	2–>64	8	8
Azithromycin	8–>128	32	128
Erythromycin	2–128	16	16
Clindamycin	0.03–0.5	0.12	0.5
S. pyogenes (constitutive ErmB) [6]			
ABT-773	0.5–8		
Clarithromycin	>128		
Azithromycin	>128		
Erythromycin	>128		
Clindamycin	128–>128		
S. aureus (macrolide S) [16]			
ABT-773	0.03	0.03	0.03
Clarithromycin	0.12–0.25	0.12	0.25
Azithromycin	0.25–1	0.5	1
Erythromycin	0.25	0.25	0.25
Clindamycin	0.06–0.12	0.12	0.12
S. aureus (inducible ErmA) [11]			
ABT-773	0.03–0.25	0.03	0.25
Clarithromycin	2–>128	>128	>128
Azithromycin	8–>128	>128	>128
Erythromycin	4–>128	>128	>128
Clindamycin	0.06–0.12	0.06	0.06
S. aureus (constitutive ErmA, ErmC) [18]			
ABT-773	>128	>128	>128
Clarithromycin	>128	>128	>128
Azithromycin	>128	>128	>128
Erythromycin	>128	>128	>128
Clindamycin	>128	>128	>128
S. epidermidis (macrolide S) [13]			
ABT-773	≤0.015–0.03	0.03	0.03
Clarithromycin	≤0.015–0.25	0.12	0.25
Azithromycin	≤0.12–1	0.5	1
Erythromycin	≤0.03–0.25	0.25	0.25
Clindamycin	0.06–0.12	0.06	0.12
S. epidermidis (constitutive MLS_B) [12]			
ABT-773	>128	>128	>128
Clarithromycin	>128	>128	>128
Azithromycin	>128	>128	>128
Erythromycin	>128	>128	>128
Clindamycin	>128	>128	>128
Enterococcus spp. (macrolide S) [12]			
ABT-773	≤0.015–0.03	0.03	0.03
Clarithromycin	0.12–0.5	0.25	0.5
Azithromycin	0.25–2	1	2
Erythromycin	0.06–0.5	0.25	0.5
Clindamycin	0.25–32	16	16

Contined on following page

TABLE 1—*Continued*

Species (resistance) [*n*] and antibiotic	MIC (μg/ml)		
	Range	50%	90%
Enterococcus spp. (macrolide R) [30]			
ABT-773	1–>128	16	128
Clarithromycin	>128	>128	>128
Azithromycin	>128	>128	>128
Erythromycin	>128	>128	>128
Clindamycin	>128	>128	>128
Corynebacterium spp. [18]			
ABT-773	0.002–0.5	0.015	0.5
Clarithromycin	0.008–>64	4	64
Azithromycin	0.03–>128	>128	>128
Erythromycin	0.004–>128	8	128
Clindamycin	0.12–>128	>128	>128
L. monocytogenes [24]			
ABT-773	0.03	0.03	0.03
Clarithromycin	0.12	0.12	0.12
Azithromycin	1	1	1
Erythromycin	0.12	0.12	0.12
E. coli [28]			
ABT-773	2–64	16	64
Clarithromycin	32–>128	128	>128
Azithromycin	2–16	4	16
Erythromycin	32–>128	128	>128
M. catarrhalis [17]			
ABT-773	0.06–0.12	0.12	0.12
Clarithromycin	0.12–0.25	0.12	0.25
Azithromycin	0.015–0.06	0.03	0.06
Erythromycin	0.12–0.25	0.12	0.25
Legionella spp. [11]			
ABT-773	0.5–1	0.5	1
Clarithromycin	0.06–0.12	0.12	0.12
Azithromycin	0.12–2	0.5	2
Erythromycin	0.25–1	1	1
N. gonorrhoeae [11]			
ABT-773	≤0.008–0.5	0.015	0.25
Clarithromycin	0.015–2	0.06	1
Azithromycin	0.03–0.25	0.03	012
Erythromycin	0.015–4	0.12	1
H. pylori (macrolide S) [15]			
ABT-773	0.008–0.06	0.03	0.06
Clarithromycin	0.008–0.06	0.008	0.015
Azithromycin	0.06–0.5	0.25	0.5
Erythromycin	0.06–0.25	0.12	0.25
H. pylori (macrolide R) [15]			
ABT-773	4–64	32	64
Clarithromycin	4–128	32	128
Azithromycin	128–>128	>128	>128
Erythromycin	64–>128	128	>128
M. avium (macrolide S) [13]			
ABT-773	0.5–128	32	64
Clarithromycin	0.25–32	4	16
Azithromycin	16–>256	64	>256
Erythromycin	4–>256	64	256
M. avium (macrolide R) [15]			
ABT-773	>256	>256	>256
Clarithromycin	>256	>256	>256
Azithromycin	>256	>256	>256
Erythromycin	>256	>256	>256

TABLE 2. Comparative in vitro activity of ABT-773 by broth dilution techniques[a]

Species (resistance) [*n*] and antibiotic	MIC (μg/ml)		
	Range	50%	90%
S. pneumoniae (macrolide S) [30]			
ABT-773	≤0.001–0.008	≤0.001	0.004
Clarithromycin	0.002–0.06	0.015	0.03
Azithromycin	0.004–0.25	0.06	0.12
Erythromycin	0.002–0.06	0.03	0.03
Clindamycin	0.008–0.06	0.03	0.03
Penicillin	0.004–4	0.03	4
S. pneumoniae (Mef) [23]			
ABT-773	≤0.002–0.12	0.06	0.06
Clarithromycin	0.5–8	4	4
Azithromycin	2–16	8	8
Erythromycin	2–8	4	8
Clindamycin	0.015–0.12	0.03	0.03
Penicillin	0.015–>1	1	>1
S. pneumoniae (ErmB) [31]			
ABT-773	0.004–2	0.008	0.25
Clarithromycin	2–>128	>128	>128
Azithromycin	4–>128	>128	>128
Erythromycin	2–>128	>128	>128
Clindamycin	16–>128	128	>128
Penicillin	≤0.008–4	1	4
H. influenzae [25]			
ABT-773	0.5–4	1	4
Clarithromycin	2–32	4	16
Azithromycin	0.25–2	0.5	2
Erythromycin	1–16	4	8
M. pneumoniae [8]			
ABT-773	≤0.0005		
Clarithromycin	0.001–0.004		
Azithromycin	≤0.0005		
Erythromycin	0.004–0.008		
B. burgdorferi [2]			
ABT-773	≤0.001		
Clarithromycin	0.004–0.008		
Azithromycin	≤0.001		
Erythromycin	0.008		

[a] Broth microdilution was used for *S. pneumoniae, H. influenzae,* and *M. pneumoniae*. Broth macrodilution was used for *B. burgdorferi*.

plain the activity of ABT-773 against other constitutive macrolide-lincosamide-streprogramin B (MLS_B) resistant species, such as *Corynebacterium* spp. However, ABT little to no vitro activity against constitutive MLS_B-resistant staphylococci and enterococci. The reason for different susceptibilities in various species with constitutive MLS_B resistance is unknown.

The MIC_{90}s for MefA strains of *S. pneumoniae* and *S. pyogenes* were 0.06 and 0.25 μg/ml, respectively. ABT-773 accumulates rapidly in cells of *S. pneumoniae* containing the Mef efflux pump because the high affinity of ABT-773 for ribosomes may overcome export (4) or ABT-773 may have poor affinity for the pump.

Penicillin susceptibility had no effect on susceptibility to ABT-773 for pneumococci. For macrolide-susceptible strains, the ABT-773 MIC_{90} was 0.002 μg/ml for 16 penicillin-susceptible strains and ≤0.002 μg/ml for 14 penicillin-nonsusceptible strains. The ABT-773 MIC_{90}s were 0.06, 0.06, and 0.12 μg/ml for 27 penicillin-susceptible, 19 penicillin-intermediate, and 37 penicillin-resistant strains, respectively. Each group comprised macrolide-susceptible, MefA, and ErmB isolates. The MIC_{90}s of ABT-773 in a study of 1,601 pneumococci are similar: ≤0.008, 0.03, and 0.12 μg/ml for penicillin-susceptible, -intermediate, and -resistant strains, respectively (2).

TABLE 3. Minimum bactericidal activities of ABT-773[a]

Species	ABT-773		Erythromycin		Ciprofloxacin	
	MIC	MBC	MIC	MBC	MIC	MBC
S. pneumoniae ATCC 6303						
No serum	0.002	0.004	0.03	0.12	1	1
Serum	0.004	0.008	0.06	2	1	2
H. influenzae 1882						
No serum	0.5	1	4	8	0.004	0.008
Serum	0.5	1	1	2	0.004	0.004
M. catarrhalis 2604						
No serum	0.25	0.5	0.25	0.5	0.12	0.12
Serum	0.12	0.12	0.12	0.12	0.12	0.12
S. aureus ATCC 6538P						
No serum	0.03	>1	0.25	>8	0.12	0.25
Serum	0.015	>1	0.03	>1	0.12	0.5

[a] In micrograms per milliliter. The serum was 50% human serum.

ABT-773 was fourfold less active than clarithromycin against macrolide-susceptible strains of *H. pylori* and *M. avium,* with MIC_{90}s of 0.06 and 64 μg/ml, respectively. Point mutations in the 23S rRNA at residues A2058 and A2059 (*E. coli* numbering) reduce macrolide binding to ribosomes and result in resistance in *H. pylori* and *M. avium* (8, 16). ABT-773 was significantly less active against *H. pylori* strains having A2058G or A2059G mutations and against *M. avium* strains having mutations at A2058 than against the corresponding susceptible strains. These results confirmed the importance of A2058 and A2059 for ABT-773 binding to ribosomes (4).

Minimum bacterial concentrations (MBCs) were determined in conjunction with broth microdilution MICs for four strains (5, 9). ABT-773 is 87 to 96% bound to human plasma proteins. The MICs and MBCs were also done in medium containing 50% (vol/vol) human serum (Scantibodies Laboratory, Inc., Santee, Calif.) which had been heated for 1 h at 56°C (Table 3). ABT-773 was bactericidal for single strains of *H. influenzae, S. pneumoniae,* and *M. catarrhalis* in medium alone or in medium containing 50% human serum, since the MBCs were no more than twofold higher than the corresponding MICs. In contrast, ABT-773 was bacteriostatic for a strain of *S. aureus*.

MBC results were confirmed by time-kill analysis using ABT-773 at four times the MIC (Fig. 1) (5). For *H. influenzae, S. pneumoniae,* and *M. catarrhalis* in medium alone or in medium containing 50% human serum, the number of viable cells remaining after 24 h of incubation with ABT-773 was reduced by at least 99.9% from the number of viable cells present in the initial inocula. For *S. aureus*, ABT-773 caused less than a 10-fold loss of viable cells after 24 h of incubation.

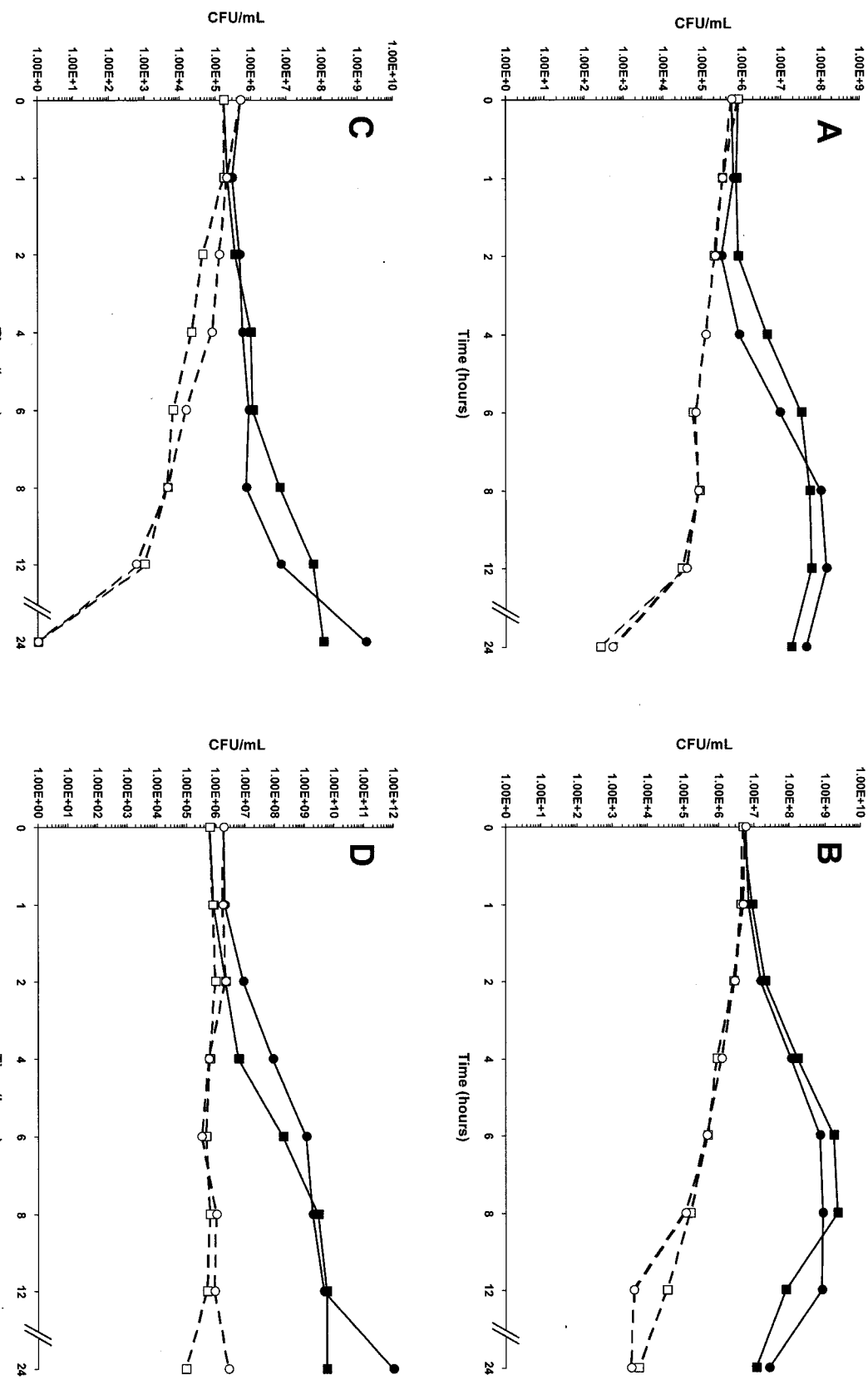

FIG. 1. Time-kill analysis of ABT-773 at antibiotic concentrations equal to four times the MIC against *S. pneumoniae* ATCC 6303 (A), *H. influenzae* 1882 (B), *M. catarrhalis* 2604 (C), and *S. aureus* ATCC 6538P (D); MICs are presented in Table 4. ■, drug-free control in medium; □, ABT-773 at four times the MIC in medium; ●, drug-free control in medium containing 50% human serum, ○, ABT-773 at four times the MIC in medium containing 50% human serum.

The overall antibacterial activity of ABT-773 was more potent and included a broader spectrum of key respiratory tract pathogens, including multidrug-resistant, atypical, and intracellular pathogens, than those of clarithromycin, azithromycin, and erythromycin. In addition, ABT-773 had comparable or improved activity against non-respiratory-tract pathogens. The improved potency is due to the greater affinity of ABT-773 to macrolide-susceptible ribosomes, resulting in rapid accumulation in bacterial cells and slower dissociation from the ribosome (4). ABT-773 retained potent antibacterial activity against macrolide-resistant pneumococcal isolates. The MIC_{90} for the 53 macrolide-resistant pneumococci tested was 0.12 μg/ml, and this activity derives from affinity for methylated streptococcal ribosomes, no or poor induction of ErmB, and the ability to overcome Mef efflux (4).

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