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In Vitro Activities of Cethromycin (ABT-773), a New Ketolide, against *Streptococcus pneumoniae* Strains That Are Not Susceptible to Penicillin or Macrolides

Edward O. Mason, Jr.,[1,2]* Linda B. Lamberth,[2] Ellen R. Wald,[3] John S. Bradley,[4] William J. Barson,[5,6] Sheldon L. Kaplan,[1,2] and the U.S. Pediatric Multicenter Pneumococcal Surveillance Group

Department of Pediatrics, Baylor College of Medicine,[1] and Texas Children's Hospital,[2] Houston, Texas; University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania[3]; Univeristy of California San Diego and the Children's Hospital of San Diego, San Diego, California[4]; and Ohio State University College of Medicine[5] and Children's Hospital of Columbus,[6] Columbus, Ohio

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Pneumococcal resistance to antimicrobials presents problems to physicians for empirical treatment of acute otitis media (AOM). Three hundred thirty-three isolates of *Streptococcus pneumoniae* selected for nonsusceptibility to penicillin (MIC >0.1 μg/ml) from the middle ear ($n = 325$) or mastoid ($n = 8$) of children seen between 1994 and 2000 at four children's hospitals in the United States were tested by broth microdilution for susceptibility to nine antibiotics. Using NCCLS 2002 breakpoints, resistance to the following drugs was as indicated: amoxicillin, 1%; azithromycin, 71%; cefprozil, 71%; ceftriaxone, 2%; cefdinir, 98%; erythromycin, 70%; levofloxacin, 0%; and trimethoprim-sulfamethoxazole, 93%. Of the penicillin- and erythromycin-nonsusceptible isolates, 97% were inhibited by cethromycin (ABT-773) and 83% were inhibited by telithromycin at a concentration of ≤0.125 μg/ml. Macrolide resistance among penicillin-nonsusceptible pneumococci increased from 44 to 80% in the 6 years of the study from which the isolates were selected; however, the proportion of isolates with M or MLS_B phenotypes remained constant over the time period (53 and 18%, respectively). Prior treatment with a macrolide or clindamycin alone or in combination with a β-lactam resulted in 94 or 85% of isolates causing infections being macrolide and or clindamycin resistant. No prior individual macrolide (azithromycin, erythromycin, or clarithromycin) resulted in more macrolide resistance or in a more prevalent resistance phenotype. The ketolides appear to be active antimicrobials against penicillin- and macrolide-resistant pneumococci.

The treatment of acute otitis media (AOM) continues to be a challenge to practitioners (16). Resistance to β-lactam and macrolide antibiotics has increased over the last 6 years and has complicated empirical therapy for nonmeningeal infections, including otitis (9, 10). Increases in macrolide resistance, often linked to penicillin nonsusceptibility, further complicate effective empirical therapy. Macrolide resistance is most often mediated by one of two mechanisms: an efflux pump (M phenotype) which confers resistance to macrolides but not clindamycin, or methylation of an adenine at the antibiotic binding site on the 50s ribosomal subunit (macrolide-lincosamide-streptogramin B [MLS_B] phenotype). The MLS_B phenotype is resistant to all the macrolides, lincosamides (clindamycin), and streptogramin B antibiotics. The prevalence of macrolide-resistant *Streptococcus pneumoniae* from middle ear infections varies geographically (5, 8). Macrolide resistance among isolates of *S. pneumoniae* recovered from children with OM from our study group during the last 6 years is approximately 51%, and clindamycin resistance for the same period is 9%. Cethromycin is a new ketolide with a broad spectrum of in vitro antibiotic activity against most β-lactam-, macrolide-, and lincosamide-resistant strains of *S. pneumoniae*. The present study examined the susceptibility of penicillin-nonsusceptible middle ear isolates of *S. pneumoniae* to 2 ketolides (cethromycin and telithromycin) and other antimicrobials used in therapy of OM.

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MATERIALS AND METHODS

Isolates of S. *pneumoniae* recovered from the middle ear of patients with OM at four children's medical centers in the United States since 1994 were selected on the basis of nonsusceptibility to penicillin (MIC > 0.1 μg/ml). Isolates were selected to include all penicillin-resistant strains (for 253 strains, MIC > 1 μg/ml) and the penicillin-intermediate strains for which the MICs of penicillin are higher ($n = 59$ for MIC = 1 μg/ml, $n = 19$ for MIC = 0.5 μg/ml, and $n = 1$ each for MICs of 0.25 and 0.125 μg/ml) The isolates had been previously confirmed as *S. pneumoniae* by colonial and microscopic morphology, alpha hemolysis on sheep blood agar, bile solubility, and/or susceptibility to optochin. All isolates were stored in the Infectious Diseases Laboratory at Texas Children's Hospital at −80°C. Serogrouping or serotyping was performed using antisera from Statens Seruminstitut, Copenhagen, Denmark.

All strains were tested for susceptibility to cethromycin and telithromycin, (Abbott Laboratories, Abbott Park, Ill.), amoxicillin, azithromycin, cefdinir, cefprozil, ceftriaxone, erythromycin, levofloxacin, penicillin, and trimethoprim-sulfamethoxazole (U.S. Pharmacopeia, Rockville, Md.), by the NCCLS broth microdilution method using an inoculum of 5×10^5 CFU per well (13). Inoculum size was confirmed by quantitative culture for each test run. Mueller-Hinton broth supplemented with 5% lysed horse blood was used as the test medium. The MIC was determined following incubation at 35°C for 24 h and interpreted by NCCLS breakpoints (12). *S. pneumoniae* ATCC 49619 was tested in each batch as a control. Phenotypic determination of macrolide resistance was determined

* Corresponding author. Mailing address: Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Texas Medical Center, Houston, TX 77030. Phone: (832) 824-4330. Fax: (832) 825-4347. E-mail: emason@bcm.tmc.edu.

TABLE 1. Susceptibility to selected antimicrobials of penicillin-nonsusceptible *S. pneumoniae* determined by NCCLS interpretive standards

Antibiotic	MIC breakpoints (μg/ml)[a]	Susceptibility based on MIC (%)		
		Susceptible	Intermediate	Resistant
Azithromycin	S ≤ 0.5; I = 1; R ≥ 2	96 (28.8)	0 (0)	237 (71.2)
Erythromycin	S ≤ 0.25; I = 5; R ≥ 1	96 (28.8)	5 (1.5)	232 (69.7)
Amoxicillin	S ≤ 2; I = 4; R ≥ 8	266 (79.9)	64 (19.2)	3 (0.9)
Ceftriaxone	S ≤ 1; I = 2; R ≥ 4	299 (89.8)	29 (8.7)	5 (1.5)
Cefprozil	S ≤ 2; I = 4; R ≥ 8	19 (5.7)	78 (23.4)	236 (70.9)
Cefdinir	S ≤ 0.5; I = 1; R ≥ 2	5 (1.5)	1 (0.3)	327 (98.2)
Levofloxacin	S ≤ 2; I = 4; R ≥ 8	332 (99.7)	1 (0.3)	0 (0)
TMP/SMZ[b]	S ≤ 0.5; I = 1–2; R ≥ 4	5 (1.5)	17 (5.1)	311 (93.4)

[a] Per NCCLS.
[b] TMP/SMZ, trimethoprim-sulfamethoxazole (ratio 1:20); results for the trimethoprim component are reported.

using erythromycin and clindamycin disks placed 15 to 20 mm apart on a Mueller-Hinton agar plate supplemented with 5% sheep blood (15). Resistance to both antibiotics was designated the MLS_B phenotype; resistance to erythromycin and susceptibility to clindamycin was designated the M phenotype.

Patient demographics and prior antibiotic therapy was obtained by retrospective review of the database of the United States Multicenter Pneumococcal Surveillance Study (11). Statistical calculations were performed using True Epistat (Epistat Services, Richardson, Tex.). Dichotomous variables were analyzed using the Fishers exact test.

RESULTS

Of the 333 penicillin-nonsusceptible isolates of *S. pneumoniae* selected from strains submitted between 1994 and 2000, 80 (24%) were penicillin intermediate (MIC between 0.1 and 1 μg/ml) and 253 (76%) were penicillin resistant (MIC >1 μg/ml). The majority (n = 325) were isolated from the middle ear, and 8 isolates were taken from mastoid tissue. The children ranged in age from 16 days to 11 years, with a median age of 1.24 years. Fifty-nine percent were male.

Table 1 shows the susceptibility of penicillin-nonsusceptible *S. pneumoniae* to selected antimicrobials determined by 2002 NCCLS breakpoints. Of the penicillin-nonsusceptible isolates, 0.9% were resistant and 19.2% were intermediate to amoxicillin and 1.5% were resistant and 8.75% were intermediate to ceftriaxone. In contrast, 94% of isolates were nonsusceptible to cefprozil and 99% were nonsusceptible to cefdinir or trimethoprim-sulfamethoxazole. All but one (levofloxacin-intermediate) isolate was susceptible to levofloxacin. Seventy-one percent of isolates were nonsusceptible to erythromycin and azithromycin. While breakpoints for the two ketolides have not been established, for only two strains were the MICs ≥1 μg/ml (for one strain the MIC was 1 μg/ml to both ketolides, and for one strain the MIC was 2 μg/ml to both ketolides). The MIC at which 50% of strains tested are inhibited (MIC_{50}), MIC_{90}, and the MIC range for all the antibiotics tested against the penicillin-nonsusceptible pneumococci can be seen in Table 2. All the penicillin-nonsusceptible isolates of *S. pneumoniae* were inhibited by the two ketolides cethromycin and telithromycin (MICs of ≤2.0 μg/ml); 97% were inhibited by cethromycin, and 87% were inhibited by telithromycin at a concentration of 0.125 μg/ml. Amoxicillin and ceftriaxone were the most active β-lactam antibiotics for penicillin-nonsusceptible isolates. The MIC_{90}s of cefdinir and cefprozil were 16 and 32 μg/ml for penicillin-intermediate and resistant strains, respectively.

Table 3 shows the MIC_{90} and MIC range for antimicrobials when the isolates were categorized as erythromycin susceptible or nonsusceptible. Seventy one per cent (71%) of these penicillin-nonsusceptible pneumococci were nonsusceptible to erythromycin and azithromycin; of these nonsusceptible stains 76% were the M phenotype and 24% were the MLS_B phenotype. Cethromycin and telithromycin also inhibited 100% of erythromycin susceptible isolates at a concentration of 0.125 μg/ml. For erythromycin-nonsusceptible isolates, cethromycin

TABLE 2. Summary of MICs[b] for penicillin-intermediate and -resistant isolates

Antibiotic	MICs (μg/ml)					
	Penicillin-intermediate strains (n = 80)			Penicillin-resistant strains (n = 253)		
	50%	90%	Range	50%	90%	Range
Cethromycin	0.125	0.125	0.125–2	0.125	0.125	0.125–1
Azithromycin	4	128	0.125–128	16	128	0.125–128
Erythromycin	2	128	0.125–128	4	128	0.125–128
Telithromycin	0.125	0.125	0.125–2	0.125	0.125	0.125–1
Amoxicillin	0.5	1	0.125–4	1	4	0.125–8
Ceftriaxone	0.25	0.5	0.125–2	0.25	2	0.125–4
Cefprozil	4	16	0.125–128	8	32	0.125–128
Cefdinir	8	16	0.125–64	8	32	0.125–128
Levofloxacin	0.5	1	0.25–4	0.5	1	0.25–2
TMP/SMZ[a]	32	32	0.06–32	32	32	0.5–32

[a] TMP/SMZ, trimethoprim-sulfamethoxazole (ratio 1:20); results for the trimethoprim component are reported.
[b] 50%, MIC_{50}; 90%, MIC_{90}.

TABLE 3. Summary of MICs for erythromycin-susceptible and -nonsusceptible isolates

Antibiotic	MICs[b] (μg/ml)			
	Erythromycin-susceptible strains (n = 97)		Erythromycin-nonsusceptible strains (n = 236)	
	90%	Range	90%	Range
Cethromycin	0.125	0.125	0.125	0.125–2
Azithromycin	0.125	0.125–2	128	2–128
Telithromycin	0.125	0.125	0.125	0.125–2
Amoxicillin	4	0.125–4	4	0.125–8
Ceftriaxone	2	0.125–2	1	0.125–4
Cefprozil	32	0.125–64	32	0.25–128
Cefdinir	64	0.125–64	32	1–128
Levofloxacin	1	0.25–4	1	0.25–2
TMP/SMZ[a]	32	0.06–32	32	1–32

[a] TMP/SMZ, trimethoprim-sulfamethoxazole (ratio 1:20); results for the trimethoprim component are reported.
[b] 90%, MIC_{90}.

inhibited 97% of isolates at an MIC of ≤0.125 μg/ml and telithromycin inhibited 83% of isolates at this concentration. Macrolide resistance increased from 44 to 80% in the 6 years of the study during which the isolates were selected; however the proportion of isolates with M or MLS_B phenotypes remained constant over the time period (53 and 18%, respectively).

A medication history within the 30 days prior to infection was available for 331 of the 333 children (Table 4). Two hundred seventy (82%) had received a prior antibiotic; 190 (70%) had strains that were resistant to a macrolide and or clindamycin. When the children had received a prior β-lactam alone (n = 178), 67% had a strain that was resistant to a macrolide or clindamycin. Seventy-three (22%) received a macrolide alone or in combination with another antibiotic (in all cases a β-lactam); 66% of these children had infections caused by strains that were resistant to a macrolide (M phenotype) and 19% of these children had infections resistant to a macrolide and clindamycin (MLS_B phenotype). Prior treatment with a macrolide or clindamycin alone resulted in 94% of isolates causing infections being a macrolide and or clindamycin resistant isolate. No prior specific macrolide (azithromycin, erythromycin, or clarithromycin) resulted in more macrolide resistance or in a more prevalent resistance phenotype. Macrolide resistance and macrolide resistance phenotypes did not correlate with the age of the children with OM. Ninety-seven percent (322 of 333) of the isolates from the middle ear or mastoid were serogroups or serotypes represented in the heptavalent conjugated pneumococcal vaccine.

DISCUSSION

Pneumococcal resistance to the β-lactam and macrolide antibiotics continues to increase. Doern and colleagues have documented macrolide nonsusceptibility rising from 10.3% in 1994 and 1995 to 26% in 1999 and 2000 in the United States (4). Macrolide resistance among isolates of *S. pneumoniae* derived from invasive infections increased from16 to 32% between 1994 and 1999 in Atlanta, Ga.; however, the prevalence of the MLS_B phenotype remained stable over the 6 years of the study (7). Clindamycin therapy of AOM caused by *S. pneumoniae* in the presence of macrolide resistance remains a viable alternative in the United States, because the predominant mechanism of macrolide resistance is the macrolide efflux pump (M phenotype), in contrast to Europe, where the MLS_B phenotype predominates (2, 6, 8). Cethromycin is a new ketolide with in vitro antibacterial activity against many gram-positive and gram-negative bacteria causing respiratory infections (3). The ketolides are active in vitro (MIC ≤ 4 μg/ml) against erythromycin-nonsusceptible *S. pneumoniae* (3), regardless of the macrolide resistance mechanism (8). In children, pneumococcal antibiotic resistance is more prevalent in

TABLE 4. Effects of antibiotic treatment in the 30 days prior to infection and recovery of macrolide-resistant isolates from patients with middle ear infection[a]

Prior antibiotics	No. (%) macrolide resistant		No. (%) macrolide susceptible
	M phenotype	MLS_B phenotype	
None (n = 58)[c]	32 (54)	10 (17)	16 (28)
Any (n = 270)[b]	143 (53)	47 (17)	80 (30)
β-Lactam only (n = 178)	90 (51)	28 (16)	60 (34)
Macrolide with or without β-lactam (n = 73)	48 (66)	14 (19)	11 (15)
Macrolide or clindamycin alone (n = 35)	25 (71)	8 (23)	2 (6)

[a] One isolate resistant to erythromycin, mechanism unknown.
[b] Two isolates, unknown prior antibiotics; two isolates resistant to erythromycin, mechanism unknown.
[c] No prior antibiotics versus any prior antibiotic, P = NS; no prior antibiotics versus β-lactam only, P = NS; no prior antibiotics versus macrolide with or without β-lactam, P = 0.09 OR 0.466 95% CI = 0.180 to 1.193; no prior antibiotics versus macrolide or clindamycin alone, P = 0.01 OR = 0.159, 95% CI = 0.039 to 0.805; β-lactam only versus macrolide with or without β-lactam, P = 0.003 OR = 0.349 95% CI = 0.16 to 0.745; β-lactam only versus macrolide or clindamycin alone, P = 0.0004 OR = 0.119 95% CI = 0.034 to 0.535.

isolates obtained from the middle ear compared to systemic isolates, making therapy with an oral agent more difficult. Using middle ear isolates that were selected for penicillin nonsusceptibility, we were able to test the in vitro performance of the ketolides and compare them to other antibiotics used in therapy of OM. It should be noted that the poor activity of cefdinir and cefprozil in this study, compared to other published data (4), is due, in large part, to the selection of penicillin-intermediate strains for which the MICs were high (0.5 and 1.0 μg/ml).

Several studies have linked macrolide use with increased isolation of macrolide-resistant *S. pneumoniae*, but these studies were not designed to establish actual treatment with a specific antibiotic prior to infection (1, 7, 14). Prior antibiotic therapy with any agent is a known risk factor for isolation of a penicillin-resistant pneumococcus (11), and penicillin resistance is often linked to resistance to macrolides, tetracyclines, and chloramphenicol (2). We were able to ascertain the specific antimicrobial used in prior therapy (within 30 days) and correlate it with the isolation of pneumococci resistant to macrolides. The pneumococci for this study were picked for nonsusceptibility to penicillin, and accordingly, macrolide resistance was expected to be high. However, it was surprising to see that prior macrolide therapy, or macrolide therapy in combination with a β-lactam, was associated with recovery of, respectively, only 15 or 6% of macrolide-susceptible isolates causing OM.

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