ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2004, p. 605–607 Vol. 48, No. 2
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.2.605–607.2004

Activities of Cethromycin and Telithromycin against Recent North American Isolates of *Streptococcus pneumoniae*

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Received 16 May 2003/Returned for modification 1 September 2003/Accepted 19 October 2003

The in vitro activities of two investigational ketolides, cethromycin (formerly ABT-773) and telithromycin, were determined for a selected group of 312 *Streptococcus pneumoniae* isolates from a national surveillance program. The MIC of cethromycin at which 50% of the isolates were inhibited was 0.008 μg/ml, and the MIC at which 90% of the isolates were inhibited was 0.06 μg/ml; the corresponding values for telithromycin were ≤0.015 and 0.25 μg/ml, respectively. For six quinupristin-dalfopristin-resistant strains, the cethromycin MICs were 0.25 to 16 μg/ml and the telithromycin MICs were 1 to 4 μg/ml. However, there was only 0.3% resistance to telithromycin.

The prevalence of antimicrobial agent resistance among both invasive and respiratory isolates of *Streptococcus pneumoniae* has increased substantially throughout the world in the past decade (2, 6, 8, 25). In addition to resistance to penicillin, older-generation cephalosporins, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole, resistance to extended-spectrum cephalosporins and fluoroquinolones has been noted (3, 4, 6, 10, 25). Two types of resistance to macrolides have been demonstrated, one that affects both macrolides and lincosamides by virtue of structural modification of the ribosomal target site by methylation [MLSB-type resistance mediated primarily by *erm*(B)] and one that affects only the macrolides [M phenotype encoded by *mef*(A)] due to an efflux pump (11, 23, 24). Both mechanisms of macrolide resistance have been associated with therapeutic failures of pneumococcal infections (12, 13). Ketolides represent a new structural modification of the macrolide structure (a ketone group replacing the cladinose moiety at the 3 position of the erythronolide A ring), with the resulting addition of a second ribosomal binding site at domain V as well as at domain II of the 23S RNA subunit (9). This structure results in good activity of ketolides against many macrolide- and lincosamide-resistant gram-positive bacteria (9, 14, 18, 20).

The Centers for Disease Control and Prevention has performed intensive surveillance in North America since 1994 for resistance among invasive pneumococcal isolates through its Active Bacterial Core Surveillance of the Emerging Infections Program (11, 25). In the years 1994 to 1996, nine sites located in various areas of the United States and Canada collected pneumococcal clinical isolates and patient data; from 1997 to 2000, seven of the sites continued with the surveillance program. A group of 312 isolates was selected for assessment of the in vitro activities of two investigational ketolide antibiotics intended for therapy of pneumococcal respiratory infections. These isolates included 162 isolates that reflected resistance mechanisms affecting most antimicrobial agent classes in current clinical use (e.g., 70 resistant to penicillin, 32 to cefotaxime, 93 to erythromycin, 47 to clindamycin, 6 to quinupristin-dalfopristin, 18 to levofloxacin, 56 to tetracycline, and 111 to trimethoprim-sulfamethoxazole), and an additional 150 susceptible isolates that were randomly selected from among the Active Bacterial Core Surveillance isolate collection of 1994 to 1998; the selection was weighted to represent equally all of the surveillance sites.

The ketolides examined in this study included cethromycin (formerly ABT-773; kindly provided by Abbott Laboratories, North Chicago, Ill.) and telithromycin (formerly HMR 3647; kindly provided by Aventis Pharmaceuticals, Romainville, France). Reagent powders of penicillin, cefotaxime, erythromycin, clindamycin, and quinupristin-dalfopristin were provided by their manufacturers or obtained from Sigma Chemical Company. The MICs of the ketolides and the comparative agents were determined by using the broth microdilution susceptibility test method recommended by the NCCLS (16). This method included use of cation-adjusted Mueller-Hinton broth supplemented with 3% lysed horse blood as the test medium,

TABLE 1. MICs of cethromycin, telithromycin, and comparative agents against a collection of 312 North American *S. pneumoniae* invasive clinical isolates

Antimicrobial agent	MIC (μg/ml)			% Resistant[a]
	50%	90%	Range	
Cethromycin	0.008	0.06	≤0.004–16	NA[b]
Telithromycin	≤0.015	0.25	≤0.015–4	0.3
Erythromycin	0.06	32	≤0.015–>64	29.8
Clindamycin	0.06	32	≤0.015–>64	15.1
Penicillin	0.03	4	≤0.015–8	22.4
Cefotaxime[c]	0.03	4	≤0.015–16	10.3

[a] Resistance defined by approved NCCLS breakpoints (17), including recently decided telithromycin breakpoints (susceptible, <1 μg/ml; intermediate, 2 μg/ml; and resistant, >4 μg/ml; T. Dooley, personal communication).
[b] NA, not applicable; approved breakpoints do not yet exist for cethromycin.
[c] Cefotaxime resistance defined in terms of NCCLS nonmeningitis breakpoints (17).

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TABLE 2. Cethromycin and telithromycin MICs displayed according to macrolide, lincosamide, and streptogramin susceptibility

Phenotype and drug[a]	No. of isolates for which the ketolide MIC (μg/ml) was:										
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
Ery^s and Cli^s											
Cethromycin	68										
Telithromycin	67	1									
Ery^r and Cli^s (M phenotype)											
Cethromycin	12	9	9	12		1					
Telithromycin				11	12	18					
Ery^r and Cli^r (MLSB phenotype)											
Cethromycin	27	9		1	1	1	6				
Telithromycin	9	23	5		6	2	1				
Q-D^r											
Cethromycin					1	3	1				1
Telithromycin							4	1	1		

[a] Ery, erythromycin; Cli, clindamycin; Q-D, quinupristin-dalfopristin.

an inoculum density of 5×10^5 CFU/ml, and incubation at 35°C in ambient air for 20 to 24 h prior to visual determination of the MICs. *S. pneumoniae* strain ATCC 49619 was tested for quality control purposes.

PCR tests were performed for *erm*(B) and *mef*(A) genes on 11 isolates selected because of elevated ketolide MICs and on 8 representative ketolide-susceptible isolates. Oligonucleotide primers were used to amplify a 1.7-kbp segment containing the *mef*(A) or *mef*(E) gene (23) or a 639-bp segment containing the *erm*(B) gene fragment (22). Positive controls for each primer pair and a negative control (*S. pneumoniae* strain ATCC 49619) were included. In addition, an internal control consisting of primers for RR142, a 400-bp conserved region of *S. pneumoniae* (24), was employed to demonstrate adequate DNA for amplification and the absence of inhibitors.

Both cethromycin and telithromycin MICs were generally very low for this selected group of pneumococcal strains (Table 1) and especially for the erythromycin-susceptible strains examined (MICs of both ketolides for these strains were ≤0.03 μg/ml) (Table 2). However, erythromycin-resistant strains were associated with slightly elevated cethromycin and telithromycin MICs, as depicted in Table 2. Indeed, the modal MICs of the two ketolides were 3 to 5 dilutions higher for the M phenotype isolates than for the fully susceptible or the MLSB phenotype strains. Perhaps the minimal effect of MLSB resistance on cethromycin and telithromycin MICs is due to the second ribosomal binding site of the ketolides (8). The activities of both ketolides were most influenced by resistance to the streptogramin antibiotic, quinupristin-dalfopristin (Table 2). For six quinupristin-dalfopristin-resistant strains, cethromycin MICs were 0.25 to 16 μg/ml (only one strain was associated with a cethromycin MIC of 16 μg/ml; all others were ≤1 μg/ml), and telithromycin MICs were 1 to 4 μg/ml. This result translated to only 0.3% (1 of 312) resistance to telithromycin based upon the recently decided NCCLS breakpoints for pneumococci (susceptible, MIC of ≤1 μg/ml; intermediate, MIC of 2 μg/ml; and resistant, MIC of ≥4 μg/ml) (T. Dooley, personal communication). With the exception of the six streptogramin-resistant strains, our findings are consistent with earlier reports (1, 5, 14, 18, 19, 20, 21) that indicated that both cethromycin and telithromycin were very active against pneumococcal strains of the efflux or MLSB phenotypes, although the MICs reported in those studies did not exceed 2 μg/ml, and telithromycin MICs usually did not exceed 1 μg/ml (1, 5, 20). PCR analysis of the six streptogramin-resistant strains with elevated cethromycin MICs did not reveal the presence of either *erm*(B) or *mef*(A) sequences. Thus, the mechanism responsible for the elevated ketolide MICs among this subset of our strains was neither of the most common macrolide resistance mechanisms. It is possible that these strains contained a different *erm* or *mef* gene or one or more mutations in the genes that encode the structure of the 23S ribosomal subunits such as the L22 or L4 proteins (7, 15, 21).

These in vitro data suggest the potential utility of cethromycin or telithromycin in the therapy of drug-resistant pneumococcal infections, depending upon the pharmacokinetic and pharmacodynamic properties as well as the safety profiles of the drugs in humans.

This study was supported in part by grants from Abbott Laboratories and by Aventis Pharmaceuticals.

REFERENCES

1. **Barry, A. L., P. C. Fuchs, and S. D. Brown.** 1998. Antipneumococcal activities of a ketolide (HMR 3647), a streptogramin (quinupristin-dalfopristin), a macrolide (erythromycin), and a lincosamide (clindamycin). Antimicrob. Agents Chemother. **42:**945–946.
2. **Butler, J. C., J. Hofmann, M. S. Cetron, J. A. Elliott, R. R. Facklam, and R. F. Breiman.** 1996. The continued emergence of drug-resistant *Streptococcus pneumoniae* in the United States: an update from the Centers for Disease Control and Prevention's pneumococcal surveillance system. J. Infect. Dis. **174:**986–993.
3. **Chen, D. K., A. McGeer, J. C. de Azavedo, and D. E. Low.** 1999. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. N. Engl. J. Med. **341:**233–239.
4. **Davidson, R., R. Cavalcanti, J. L. Brunton, D. J. Bast, J. C. S. de Azavedo, P. Kibsey, C. Fleming, and D. E. Low.** 2002. Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. N. Engl. J. Med. **346:**747–750.
5. **Davies, T. A., L. M. Kelly, M. R. Jacobs, and P. C. Appelbaum.** 2000. Antipneumococcal activity of telithromycin by agar dilution, microdilution, E test, and disk diffusion methodologies. J. Clin. Microbiol. **38:**1444–1448.
6. **Doern, G. V., A. B. Brueggemann, H. Huynh, E. Wingert, and P. Rhomberg.** 1999. Antimicrobial resistance with *Streptococcus pneumoniae* in the United States, 1997–98. Emerg. Infect. Dis. **5:**757–765.
7. **Farrel, D. J., S. Douthwaite, I. Morrissey, S. Bakker, J. Poehlsgaard, L. Jakobsen, and D. Felmingham.** 2003. Macrolide resistance by ribosomal

mutation in clinical isolates of *Streptococcus pneumoniae* from the PROTEKT 1999–2000 study. Antimicrob. Agents Chemother. **47:**1777–1783.

8. **Feikin, D. R., A. Schuchat, M. Kolczak, N. L. Barrett, L. H. Harrison, L. Lefkowitz, A. McGeer, M. M. Farley, D. J. Vugia, C. Lexau, K. R. Stefonek, J. E. Patterson, and J. H. Jorgensen.** 2000. Mortality from invasive pneumococcal pneumonia in the era of antibiotic resistance, 1995–1997. Am. J. Publ. Health **90:**223–229.
9. **Hansen, L., H. Mauvais, and S. Douthwaite.** 1999. The macrolide-ketolide antibiotic binding site is formed by structures in domain II and V of 23S ribosomal RNA. Mol. Microbiol. **31:**623–631.
10. **Ho, P. L., T. L. Que, D. N. Tsang, T. K. Ng, K. H. Chow, and W. H. Seto.** 1999. Emergence of fluoroquinolone resistance among multiply resistant strains of *Streptococcus pneumoniae* in Hong Kong. Antimicrob. Agents Chemother. **43:**1310–1313.
11. **Hyde, T. B., K. Gay, D. S. Stephens, D. J. Vugia, M. Pass, S. Johnson, N. L. Barrett, W. Schaffner, P. R. Cieslak, P. S. Maupin, E. R. Zell, J. H. Jorgensen, R. R. Facklam, and C. G. Whitney.** 2001. Macrolide resistance among invasive *Streptococcus pneumoniae* isolates. JAMA **286:**1857–1862.
12. **Kelley, M. A., D. M, Weber, P. Gilligan, and M. S. Cohen.** 2000. Breakthrough pneumococcal bacteremia in patients being treated with azithromycin and clarithromycin. Clin. Infect. Dis. **31:**1008–1011.
13. **Lonks, J. R., J. Garau, L. Gomez, M. Xercavins, A. Ochoa de Exhaguen, I. F. Gareen, P. T. Reiss, and A. A. Madeiros.** 2002. Failure of macrolide antibiotic treatment in patients with bacteremia due to erythromycin-resistant *Streptococcus pneumoniae*. Clin. Infect. Dis. **35:**556–564.
14. **Mason, E. O., Jr., L. B. Lamberth, E. R. Wald, J. S. Bradley, W. J. Barson, and S. L. Kaplan.** 2003. In vitro activities of cethromycin (ABT-773), a new ketolide, against *Streptococcus pneumoniae* strains that are not susceptible to penicillin or macrolides. Antimicrob. Agents Chemother. **47:**166–169.
15. **Musher, D. M., M. E. Dowell, V. D. Shortridge, R. K. Flamm, J. H. Jorgensen, P. Le Magueres, and K. L. Krause.** 2002. Emergence of macrolide resistance during treatment of pneumococcal pneumonia with azithromycin. N. Engl. J. Med. **346:**630–631.
16. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
17. **National Committee for Clinical Laboratory Standards.** 2003. Performance standards for antimicrobial susceptibility testing. Supplement M100-S13. National Committee for Clinical Laboratory Standards, Wayne, Pa.
18. **Nilius, A. M., M. H. Bui, L. Almer, D. Hensey-Rudloff, J. Beyer, Z. Ma, Y. S. Or, and R. K. Flamm.** 2001. Comparative in vitro activity of ABT-773, a novel antibacterial ketolide. Antimicrob. Agents Chemother. **45:**2163–2168.
19. **Nilius, A. M., D. M. Hensey-Rudloff, M. A. Reimann, and R. K. Flamm.** 2002. Comparison of selection for mutants with reduced susceptibility to ABT-773, erythromycin and rifampicin in respiratory tract pathogens. J. Antimicrob. Chemother. **49:**831–836.
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. **Shortridge, V. D., P. Zhong, Z. Cao, J. M. Beyer, L. S. Almer, N. Ramer, S. Z. Doktor, and R. K. Flamm.** 2002. Comparison of in vitro activities of ABT-773 and telithromycin against macrolide-susceptible and -resistant streptococci and staphylococci. Antimicrob. Agents Chemother. **46:**783–786.
22. **Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
23. **Tait-Kamradt, A., J. Clancy, M. Cronan, F. Dib-Haji, L. Wondrack, W. Yuan, and J. Sutcliffe.** 1997. *mefE* is necessary for the erythromycin-resistant M phenotype in *Streptococcus pneumoniae.* Antimicrob. Agents Chemother. **41:**2251–2255.
24. **Waites, K., C. Johnson, B. Gray, K. Edwards, M. Crain, W. Benjamin, Jr.** 2000. Use of clindamycin disks to predict macrolide resistance mediated by *ermB* and *mefE* in *Streptococcus pneumoniae.* J. Clin. Microbiol. **38:**1731–1734.
25. **Whitney, C. G., M. M. Farley, J. Hadler, L. H. Harrison, C. Lexau, A. Reingold, L. Lefkowitz, P. R. Cieslak, M. Cetron, E. R. Zell, J. H. Jorgensen, and A. Schuchat.** 2001. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. N. Engl. J. Med. **343:**1917–1924.