ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2008, p. 378–381 Vol. 52, No. 1
0066-4804/08/$08.00+0 doi:10.1128/AAC.01068-07

Characterization and Dynamics of Middle Ear Fluid and Nasopharyngeal Isolates of *Streptococcus pneumoniae* from 12 Children Treated with Levofloxacin▽

Todd A. Davies,[1]* Eugene Leibovitz,[2] Gary J. Noel,[1] David F. McNeeley,[1]† Karen Bush,[1] and Ron Dagan[2]

Johnson & Johnson Pharmaceutical Research and Development, LLC, Raritan, New Jersey,[1] and Pediatric Infectious Disease Unit, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel[2]

Received 13 August 2007/Returned for modification 12 September 2007/Accepted 29 October 2007

Children who had acute otitis media and were treated with levofloxacin were assessed for the emergence of fluoroquinolone-resistant *Streptococcus pneumoniae*. Nasopharynx cultures were obtained from patients at the entry to and during levofloxacin therapy. All nasopharynx isolates ($n = 59$) from 12 children were levofloxacin susceptible without *parC*/*E* or *gyrA*/*B* mutations. Pneumococcal nasopharynx persistence was not associated with levofloxacin resistance.

Acute otitis media (AOM) is the leading cause for the prescribed use of antibiotics in children (12). The primary pathogen in AOM is *Streptococcus pneumoniae* (12). The high prevalence of penicillin resistance and multidrug resistance in *S. pneumoniae* isolates has prompted the use of additional agents for the treatment of recurrent/persistent AOM in patients who fail therapy with first- and second-line agents such as β-lactams, macrolides, or sulfonamides. Fluoroquinolones, which have been used widely as an effective therapeutic agent for respiratory tract infections in adults, were studied recently in children for their safety and efficacy in the treatment of recurrent/persistent AOM (1, 2, 8).

An important consideration for the treatment of AOM is how the prescribed antibiotic affects the colonization of the nasopharynx by *S. pneumoniae*. Previous studies have shown that the use of β-lactam and macrolide agents can result in a higher incidence of posttreatment drug-resistant pneumococcal isolates in the nasopharynx than that of pretreatment isolates (4, 6, 11). These resistant pneumococci then may cause a superinfection of the middle ear fluid (MEF) (5). In this study, we examined *S. pneumoniae* isolates from MEF and nasopharynx cultures from children with recurrent/persistent AOM who were treated with levofloxacin to determine if resistance developed during treatment.

A complete description of this trial, conducted as part of a multicenter, nonrandomized, open-label study conducted in the United States, Argentina, Costa Rica, and Israel, has been published previously (1). The current study focused on isolates collected at the Israeli site following approval by the investigational review board at each institution. A data safety monitoring committee was established to monitor for any potential safety risks for children participating in this trial.

Patients (≥6 months old to <5 years old) were enrolled if (i) they had clinical signs and symptoms of AOM and (ii) they were diagnosed with either persistent disease (AOM persisting despite >72 h of appropriate antibiotic therapy) or recurrent disease (≥3 episodes of AOM in 6 months or ≥4 episodes of AOM within the previous year). Patients received 10 mg/kg of body weight of levofloxacin suspension orally twice daily for 10 days. Before dosing (pretreatment, day 1), MEF was collected for bacterial culture. Patients were clinically evaluated at three ensuing visits: 4 to 6 days after receiving the first dose of levofloxacin (on therapy [OT]), 2 to 5 days after receiving the last dose (end of therapy [EOT]), and 10 to 17 days after the last dose was received (end of study [EOS]). MEF was collected on day 1 from both ears if bilateral bulging was observed, and a second tap was mandatory at the OT visit if a positive baseline MEF culture was present. Nasopharyngeal swabs were taken at each visit to assess the presence of pneumococci. Of the 83 children enrolled at this site, 24 had pneumococci isolated from the baseline MEF, and 18 of these children met the criteria for microbiologic evaluablilty (i.e., they returned for a second evaluation and completed their protocol-defined procedures). Of these 18 children, 12 had *S. pneumoniae* organisms isolated from MEF cultures upon entry into the trial and from nasopharynx cultures on at least one occasion during therapy. These isolates were analyzed in this study. It is important to note that none of the children received prior immunization with the 7-valent conjugate pneumococcal vaccine as it was not yet licensed in Israel at the time of this study.

MICs were determined by the broth microdilution test using panels manufactured by Trek Diagnostic Systems (Cleveland, OH) according to CLSI recommendations (3). Pyrosequencing technology (Biotage, Westborough, MA) was used as described previously (10) to detect mutations in the quinolone resistance-determining region (QRDR) of *parC*, *gyrA*, *parE*, and *gyrB*, the targets of the fluoroquinolones (15). Pulsed-field gel electrophoresis (PFGE) was performed as described previously (9), and PFGE patterns were compared as described by Tenover et al. (16). Serotyping was performed by the standard

* Corresponding author. Mailing address: Johnson & Johnson Pharmaceutical Research and Development, LLC, Room B225, 1000 Route 202, Raritan, NJ 08869. Phone: (908) 707-3465. Fax: (908) 707-3501. E-mail: tdavies@prdus.jnj.com.
† Present address: Tibotec, Inc., Yardley, PA.
▽ Published ahead of print on 12 November 2007.

TABLE 1. *S. pneumoniae* isolation dates, levofloxacin MICs, and genotype

Patient	Day of study	Visit[b]	MEF culture[a]			Nasopharynx culture		
			Levofloxacin MIC (μg/ml)	PFGE type	Serotype	Levofloxacin MIC (μg/ml)	PFGE type	Serotype
1	1	PT	0.5	A	6A	0.5	A	6A
	5	OT	NA[c]	—[d]		1	A	6A
	24	EOS	NA	—		0.5	A	6A
	39	UNS	NA	—		0.5	A	6A
2	1	PT	1	W	19A	0.5	B	NT[e]
	5	OT	NA	—		0.5	C	NT
	15	EOT	NA	—		0.5	B	NT
	19	EOS	NA	—		1	D	9V
3	1	PT	0.5	E	23F	0.5	E	23F
	4	OT	NA	—		0.5	E	23F
	15	EOT	NA	—		0.5	E	23F
	25	EOS	NA	—		0.5	E	23F
4	1	PT	1	F	19F	1	F	19F
	6	OT	1	F	19F	1	F	19F
	13	EOT	NA	—		1	F	19F
	21	EOS	NA	—		0.5	G	NT
	35	UNS	NA	—		1	F	19F
5	1	PT	0.5	H	6B	NA	—	
	5	OT	NA	—		NA	—	
	25	EOS	NA	—		0.5	H	6B
	37	UNS	0.5	H	6B	NA	—	
6	1	PT	1 (right)	I	14	1	J	19F
			1 (left)	I	14	NA	—	
	13	EOT	NA	—		1	I	14
	18	EOS	NA	—		0.5	K	6B
7	1	PT	0.5	L	6A	0.5	L	6A
	23	EOS	NA	—		1	M	NT
8	1	PT	0.5	N	23F	1	N	23F
	5	OT	NA	—		1	N	23F
	12	EOT	NA	—		1	N	23F
9	1	PT	1 (right)	O	15A	0.5	O	15A
	1		1 (left)	O	15A	NA	—	
	5	OT	NA	—		1	O	15A
	12	EOT	NA	—		1	O	15A
	23	EOS	NA	—		1	O	15A
	40	UNS	NA	—		1	O	15A
10	1	PT	0.5 (right)	O1[f]	19F	1	O2[e]	19F
			1 (left)	O2[f]	19F			
	13	EOT	NA	—		1	P	11A
	21	EOS	NA	—		1	O1[f]	19F
	42	UNS	NA	—		0.5	O1[f]	19F
11	1	PT	1	Q	17F	1	Q	17F
	14	EOT	NA	—		1	R	15B
	21	EOS	NA	—		0.5	S	10A
12	1	PT	1	T	19F	1	T1	19F
	12	EOT	0.5	U	4	1	U	4
	41	UNS	NA	—		0.5	V	15B

[a] Some patients had bilateral AOM, and MEF was removed from both left and right ears.
[b] PT, pretreatment visit; UNS, unscheduled visit.
[c] NA, not applicable since no specimen was obtained.
[d] —, no PFGE data were available because *S. pneumoniae* was not isolated during that visit.
[e] NT, nontypeable as *S. pneumoniae* because cultures did not react to Omni-sera.
[f] PFGE types O1 and O2 are variants (1- to 2-band difference) of PFGE type O.

Quellung method with sera from the Statens Seruminstitut (Copenhagen, Denmark).

All *S. pneumoniae* MEF or nasopharyngeal cultures were susceptible to levofloxacin, with MICs ranging from 0.5 to 1 μg/ml (Table 1). Cultures isolated during or after levofloxacin treatment had the same levofloxacin MIC (or were within one doubling dilution) as the pretreatment isolate(s). None of the isolates had *parC*, *parE*, *gyrA*, or *gyrB* QRDR mutations known to contribute to reduced fluoroquinolone susceptibilities.

Three patients (patients 6, 9, and 10) had *S. pneumoniae* cultured from MEF from both ears at the pretreatment visit, and these isolates were the same or closely related, as determined by PFGE. Three other patients (patients 4, 5, and 12) had *S. pneumoniae* isolated from MEF obtained at some other visit in addition to that from the pretreatment visit. In two of these patients (no. 4 and 5), all MEF *S. pneumoniae* isolates were the same, as determined by PFGE, and in the other patient (no. 12), the MEF *S. pneumoniae* isolates were different (Table 1).

In nine patients (patients 1, 3, 4, and 7 to 12), the pretreatment MEF and nasopharyngeal pneumococcal cultures were identical or closely related, as shown by PFGE (Table 1). All of the nasopharyngeal cultures obtained subsequently from four (patients 1, 3, 8, and 9) of these nine patients (at the OT, EOT, or EOS visit) matched the PFGE patterns of their pretreatment MEF cultures. Subsequent nasopharyngeal cultures from six patients (patients 4, 6, 7, 10, 11, and 12) did not have the same PFGE patterns as those from their pretreatment MEF culture, indicating colonization by a new strain. Only one patient (no. 2) did not have any nasopharyngeal cultures that had PFGE patterns similar to the pretreatment MEF culture, demonstrating the simultaneous presence of two different strains in the ear and the nasopharynx.

Among the 12 children in whom persistent colonization with *S. pneumoniae* was documented, there was no phenotypic evidence of the emergence of levofloxacin resistance, as levofloxacin MICs remained between 0.5 and 1 μg/ml during treatment. *S. pneumoniae* resistance to fluoroquinolones occurs in a stepwise fashion, with the first mutation occurring typically in the QRDR of either *parC* or *gyrA* (depending on the selecting fluoroquinolone), leading to decreased fluoroquinolone susceptibility (15). A second mutation in the paralog gene usually causes a strain to become fluoroquinolone resistant (15). *S. pneumoniae* isolates with a single *parC* QRDR mutation generally have levofloxacin MICs of 1 or 2 μg/ml (10). Susceptibility testing alone may not detect isolates that gain a single QRDR mutation while the child is being treated with levofloxacin. However, genetic analysis of these isolates showed that there were no first-step mutants since QRDR mutations in the target genes were not present. Similarly, Leibovitz et al. reported that all pneumococcal isolates collected from children treated with gatifloxacin for recurrent/nonresponsive AOM were gatifloxacin susceptible (13). However, it is unknown if any of the isolates were first-step mutants, since screening for QRDR mutations was not performed.

Bacterial eradication from MEF during therapy is associated with a higher clinical success rate (7). In this study, the initial *S. pneumoniae* MEF culture was eradicated in 11 of the 12 children by the OT visit; however, six of the children did have another occurrence of otitis media after the EOT or the EOS visit. Libson et al. reported that the failure to eradicate *S. pneumoniae* from the nasopharynx by the completion of antibiotic therapy predisposed the patient to a recurrence of AOM (14). The 12 patients from our study were chosen for examination because *S. pneumoniae* organisms were isolated from the nasopharynx throughout their treatment. In the Libson et al. study recurrent AOM was usually caused by a pneumococcal isolate in the MEF that was found in the nasopharynx at the EOT visit (14). We observed a similar outcome for one of the six children (no. 5), who had another occurrence of AOM after the EOS visit and had a pneumococcal isolate cultured from the MEF that matched the nasopharynx isolate at the EOS visit. In the other five children, the MEF cultures were negative.

This small experience does not establish the fact that levofloxacin resistance may not emerge in the clinical treatment of children with recurrent/persistent AOM. However, these results clearly indicate that for the children in this study, persistent colonization of the nasopharynx was not associated with levofloxacin resistance. It is uncertain if these findings could be generalized to other patients.

This work was supported by Johnson & Johnson Pharmaceutical Research and Development, LLC.

REFERENCES

1. **Arguedas, A., R. Dagan, M. Pichichero, E. Leibovitz, J. Blumer, D. F. McNeeley, R. Melkote, and G. J. Noel**. 2006. An open-label, double tympanocentesis study of levofloxacin therapy in children with, or at high risk for, recurrent or persistent acute otitis media. Pediatr. Infect. Dis. J. **25:**1102–1109.
2. **Arguedas, A., L. Sher, E. Lopez, X. Saez-Llorens, K. Hamed, K. Skuba, and P. F. Pierce.** 2003. Open label, multicenter study of gatifloxacin treatment of recurrent otitis media and acute otitis media treatment failure. Pediatr. Infect. Dis. J. **22:**949–956.
3. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. Approved standard M7–A7. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Cohen, R., E. Bingen, E. Varon, F. de La Rocque, N. Brahimi, C. Levy, M. Boucherat, J. Langue, and P. Geslin.** 1997. Change in nasopharyngeal carriage of *Streptococcus pneumoniae* resulting from antibiotic therapy for acute otitis media in children. Pediatr. Infect. Dis. J. **16:**555–560.
5. **Dagan, R., E. Leibovitz, G. Cheletz, A. Leiberman, and N. Porat.** 2001. Antibiotic treatment in acute otitis media promotes superinfection with resistant *Streptococcus pneumoniae* carried before initiation of treatment. J. Infect. Dis. **183:**880–886.
6. **Dagan, R., E. Leibovitz, D. Greenberg, P. Yagupsky, D. M. Fliss, and A. Leiberman.** 1998. Dynamics of pneumococcal nasopharyngeal colonization during the first days of antibiotic treatment in pediatric patients. Pediatr. Infect. Dis. J. **17:**880–885.
7. **Dagan, R., E. Leibovitz, D. Greenberg, P. Yagupsky, D. M. Fliss, and A. Leiberman.** 1998. Early eradication of pathogens from middle ear fluid during antibiotic treatment of acute otitis media is associated with improved clinical outcome. Pediatr. Infect. Dis. J. **17:**776–782.
8. **Dagan, R., T. Davies, E. Leibovitz, D. F. McNeeley, K. Bush, and G. J. Noel.** 2004. Genotypic characterization of pneumococcal middle-ear fluid and nasopharyngeal isolates from infants and young children with persistent or recurrent acute otitis media treated with levofloxacin. Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. G-2082.
9. **Davies, T. A., R. Goldschmidt, S. Pfleger, M. Loeloff, K. Bush, D. F. Sahm, and A. Evangelista.** 2003. Cross-resistance, relatedness and allele analysis of fluoroquinolone-resistant United States clinical isolates of *Streptococcus pneumoniae* (1998–2000). J. Antimicrob. Chemother. **52:**168–175.
10. **Davies, T. A., Y. C. Yee, R. Goldschmidt, K. Bush, D. F. Sahm, and A. Evangelista.** 2006. Infrequent occurrence of single mutations in topoisomerase IV and DNA gyrase genes among US levofloxacin-susceptible clinical isolates of *Streptococcus pneumoniae* from nine institutions (1999–2003). J. Antimicrob. Chemother. **57:**437–442.
11. **Ghaffar, F., L. S. Muniz, K. Katz, J. Reynolds, J. L. Smith, P. Davis, I. R. Friedland, and G. H. McCracken, Jr.** 2000. Effects of amoxicillin/clavulanate or azithromycin on nasopharyngeal carriage of *Streptococcus pneumoniae*

and *Haemophilus influenzae* in children with acute otitis media. Clin. Infect. Dis. **31:**875–880.
12. **Klein, J. O.** 1994. Otitis media. Clin. Infect. Dis. **19:**823–833.
13. **Leibovitz, E., L. Piglansky, S. Raiz, D. Greenberg, K. A. Hamed, J.-M. Ledeine, J. Press, A. Leiberman, R. M. Echols, P. F. Pierce, M. R. Jacobs, and R. Dagan.** 2003. Bacteriologic and clinical efficacy of oral gatifloxacin for the treatment of recurrent/nonresponsive acute otitis media: an open label, noncomparative, double tympanocentesis study. Pediatr. Infect. Dis. J. **22:** 943–949.
14. **Libson, S., R. Dagan, D. Greenberg, N. Porat, R. Trepler, A. Leiberman, and E. Leibovitz.** 2005. Nasopharyngeal carriage of *Streptococcus pneumoniae* at the completion of successful antibiotic treatment of acute otitis media predisposes to early clinical recurrence. J. Infect. Dis. **191:**1869–1875.
15. **Pan, X.-S., J. Ambler, S. Mehtar, and L. M. Fisher.** 1996. Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae.* Antimicrob. Agents Chemother. **40:**2321–2326.
16. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.