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Bacteriologic Efficacies of Oral Azithromycin and Oral Cefaclor in Treatment of Acute Otitis Media in Infants and Young Children

RON DAGAN,[1]* EUGENE LEIBOVITZ,[1] DAN M. FLISS,[2] ALBERTO LEIBERMAN,[2] MICHAEL R. JACOBS,[3] WILLIAM CRAIG,[4] AND PABLO YAGUPSKY[5]

Pediatric Infectious Disease Unit[1] and Department of Otolaryngology,[2] Clinical Microbiology Laboratory,[5] Soroka University Medical Center, and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; Department of Pathology, Case Western Reserve University, Cleveland, Ohio[3]; and Department of Medicine, University of Wisconsin, and Williams S. Middleton Memorial Veterans Hospital, Madison, Wisconsin[4]

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A prospective, open-label, randomized study was conducted in order to determine the bacteriologic efficacies of cefaclor and azithromycin in acute otitis media (AOM). Tympanocentesis was performed on entry into the study and 3 to 4 days after initiation of treatment. Bacteriologic failure after 3 to 4 days of treatment with both drugs occurred in a high proportion of culture-positive patients, especially in those in whom AOM was caused by *Haemophilus influenzae* (16 of 33 [53%] of those treated with azithromycin and 13 of 34 [52%] of those treated with cefaclor). Although a clear correlation of the persistence of the pathogen with increased MICs of the respective drugs could be demonstrated for *Streptococcus pneumoniae*, no such correlation was found for *H. influenzae*. It is proposed that susceptibility breakpoints for *H. influenzae* should be considerably lower than the current ones for both cefaclor and azithromycin for AOM caused by *H. influenzae*.

By definition, the goal of antibiotics in the treatment of acute otitis media (AOM) is eradication of the causative organism from the middle-ear fluid. In order to reach this goal, two conditions must be met: (i) the drug should be active against the causative organisms, and (ii) the drug should reach the middle-ear fluid and maintain a sufficient concentration long enough to allow bacterial inhibition and eventual killing (11). Although these two conditions can be tested in animal models, the ultimate challenge is the eradication of the pathogens from humans and, more specifically, from infants and young children, since they constitute the majority of patients with AOM.

Unfortunately, the pharmacodynamic profiles of antimicrobial drugs (i.e., the relationship between concentrations at the site of infection over time and the antimicrobial effect) may not always be determined with accuracy in infants and young children, for obvious reasons. Thus, eradication of the pathogens must be studied in infants and children with AOM. Pathogen eradication can be assessed by performing trials in which a middle-ear fluid sample for culture is obtained immediately before antibiotic administration and a second one is obtained during the course of treatment (35). Howie and Ploussard (23) were the first to introduce this method and named it the "in vivo sensitivity test." The advantage of this method is that, with relatively few enrolled subjects, it can discriminate the effects of different drugs and predict clinical efficacy (13, 14, 23–26, 34, 35).

The recent increase in the prevalence of antibiotic-resistant *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* has important implications for the treatment of AOM and poses a challenge to clinicians (5, 7, 13, 18, 21, 25, 28, 33, 48, 49; E. Leibovitz, O. Abramson, D. Greenberg, P. Yagupsky, D. M. Fliss, A. Leiberman, R. Dagan, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. LM59, 1996). We recently demonstrated that in patients with AOM caused by *S. pneumoniae* isolates not susceptible to β-lactam antibiotics, the bacteriologic outcome was unsatisfactory in patients treated with various β-lactam antibiotics 13, 14, 32; Leibovitz et al., 36th ICAAC). The introduction of the new macrolides, such as clarithromycin, and a new azalide, azithromycin, raised new hopes for better eradication of the pathogens that cause AOM since they are active in vitro against *H. influenzae* and many β-lactam-nonsusceptible *S. pneumoniae* strains (45).

The present prospective, open-label, randomized study was therefore constructed to determine whether treatment of AOM with the new azalide drug, azithromycin, is superior to treatment with cefaclor, which was proved in previous studies to be a relatively ineffective drug against both *H. influenzae* and β-lactam-nonsusceptible *S. pneumoniae* strains in patients with AOM (14, 27, 34).

MATERIALS AND METHODS

Patients visiting the Soroka Medical Center Pediatric Emergency Room were enrolled if they (i) were 3 to 36 months old; (ii) had AOM as established on the basis of symptoms (pain in affected ear, tugging of the ear, fever, lethargy, or irritation) and signs (erythema, fullness, or bulging of the tympanic membrane); (iii) had an acute illness of ≤7 days in duration; (iv) had an intact ear drum; (v) were able to accept oral treatment (no vomiting, hemodynamically stable); and (vi) had purulent, mucopurulent, or seropurulent fluid on tympanocentesis. Patients were excluded if they had (i) received another antimicrobial agent within 72 h before enrollment (unless there was a clear clinical failure of the other antimicrobial agent); (ii) a concomitant infection requiring treatment with another systemic antimicrobial agent in addition to the study drug; (iii) chronic otitis media; (iv) an underlying condition known to compromise their ability to handle bacterial infections such as immunodeficiency, splenectomy, uncontrolled diabetes mellitus, or AIDS; and (v) a dry tap (no middle-ear fluid was obtained on tympanocentesis).

Study conduct. This was an open-label, randomized comparative study. Part of the clinical results of this study were described elsewhere (12). In the previous article we described children with AOM who received amoxicillin, cefaclor, or azithromycin. In fact, the randomization was done only for the cefaclor and azithromycin groups, while the amoxicillin group was added after the study was

* Corresponding author. Mailing address: Pediatric Infectious Disease Unit, Soroka University Medical Center, P.O. Box 151, Beer-Sheva 84101, Israel. Phone: (972-7) 640 0547. Fax: (972-7) 623 2334. E-mail: rdagan@bgumail.bgu.ac.il.

started, and thus, the amoxicillin treatment was not randomized. Therefore, in the present article we present the results of the analysis for the cefaclor and azithromycin groups only.

The trial was approved by the Soroka Medical Center and the National Ethics Committees. Written informed consent was obtained from the parents of all patients.

An otolaryngologist performed tympanocentesis for all patients at enrollment. Antisepsis of the ear canal before tympanocentesis was done with 70% alcohol, which was instilled for 1 min. After removal of the alcohol by suction and with the use of a 20-gauge (7.6- to 8.9-cm) spinal needle attached to a 1.0-ml sterilized syringe, the anteroinferior portion of the intact tympanic membrane was punctured. The fluid was immediately aspirated into the sterile syringe by suction, applied onto a sterile swab, and sent for bacteriologic culture in transport medium (MW 173 Amies medium; Transwab; Medical Wire and Equipment, Potley, United Kingdom) for processing within 12 h. After tympanocentesis, patients were randomized to receive either an oral suspension of azithromycin at 10 mg/kg of body weight in one daily dose for a total of 3 days or an oral suspension of cefaclor at 40 mg/kg/day in three divided doses for a total of 10 days.

The first follow-up visit was on day 4 or 5 (the day of enrollment was defined as day 1). A second tympanocentesis for retrieval of middle-ear fluid for culture was planned for this visit. Additional follow-up visits on days 10 and 17 ± 2 were planned. A third tympanocentesis and culture were planned for any time during follow-up if a clinical relapse occurred. Any patient with a positive culture after day 4 but before day 11 was considered to have a bacteriologic failure. For any patient with a positive culture after completion of antibiotic therapy (after day 10), the isolate was compared with the initial isolate. If the culture after day 10 yielded an organism identical to the original isolate, the patient was considered to have a bacteriologic relapse. For *S. pneumoniae* identical organisms were defined as those with the same antibiograms and serotypes, and for *H. influenzae* identical organisms were defined as those with the same biotypes and antibiograms.

All otologic evaluations were done by an otolaryngologist who was unaware of the culture results for the patient and the study drug allocation. Otologic criteria for enrollment, improvement, cure, and relapse were as follows. By definition at enrollment, initial findings always included the presence of purulent, mucopurulent, or seropurulent fluid. During follow-up, persistence of the initial fluid characteristics, coupled with signs of inflammation of the tympanic membrane, was defined as otologic failure. The presence of serous fluid with or without blood, mucoid fluid, or no fluid with signs of tympanic membrane inflammation was defined as otologic improvement. No fluid (dry tap) coupled with the absence of inflammatory signs was defined as otologic cure. Otologic relapse was defined when, after initial improvement, there was reaccumulation of pus in the middle ear and inflammation of the tympanic membrane associated with symptoms of otitis media at any time during follow-up. Children with bacteriologic failure but clinical improvement continued to receive the allocated treatment regimen; those with bacteriologic and clinical failures were switched to oral amoxicillin-clavulanate or intramuscular ceftriaxone.

Compliance was assessed by measuring the amounts of drug returned during treatment (days 4 or 5) and after treatment. The use of <80% of the planned volume was defined as noncompliance.

Bacteriology. Swabs of the middle-ear aspirate were plated onto Trypticase agar medium containing 5% sheep blood and on chocolate agar. The plates were incubated at 35°C for up to 48 h in 5% CO_2-enriched atmosphere.

Identification of *S. pneumoniae* was based on growth of alpha-hemolytic colonies, inhibition by optochin, and a positive slide agglutination test (Phadebact; Pharmacia Diagnostics, Uppsala, Sweden). Isolates were serotyped by the capsular swelling reaction by established procedures (3).

Identification of *H. influenzae* was based on the Gram staining result, growth on chocolate agar medium, failure to grow on Trypticase agar with sheep blood, and a nutritional requirement for both hemin and NAD. Isolates were serotyped with polyvalent antisera to *H. influenzae* groups a, c to f, and b (Phadebact; Pharmacia); strains that failed to agglutinate were considered untypeable. Biotyping was done with isolates from patients with bacteriologic failure and was based on the presence of urease and ornithine decarboxylase activities and the production of indole (API NH kit; bioMérieux, Lyon, France).

Identification of *M. catarrhalis* was based on the Gram staining result, a positive oxidase reaction, and a characteristic biochemical profile as determined with the API NH kit and confirmed with the NET kit (Carr Scarborough Microbiologicals, Decatur, Ga.).

Testing of *S. pneumoniae* susceptibility to penicillin and cefaclor was performed by the E-test (AB Biodisk, Solan, Sweden) on Mueller-Hinton agar supplemented with 5% sheep blood) and incubated for 18 h in 5% CO_2. *S. pneumoniae* was defined as penicillin susceptible if the MIC of penicillin was <0.1 μg/ml, intermediate if the MIC was 0.1 to 1.0 μg/ml, and resistant if the MIC was >1.0 μg/ml (38). Since we encountered technical problems with the E-test method for testing of the susceptibility of *S. pneumoniae* to azithromycin (16), the isolates were sent to the Clinical Microbiology Laboratory of the Case Western Reserve University, Cleveland, Ohio, for microdilution testing. Microdilution testing was done by the methods of the National Committee for Clinical Laboratory Standards (NCCLS) (39).

Testing of *H. influenzae* susceptibility to cefaclor and azithromycin was performed by the E-test on *Haemophilus* test medium incubated for 18 h in 5% CO_2. To validate the E-test results obtained at the Soroka Medical Center, a blinded comparison of the results of the E-test and those of the microdilution method was performed with 40 *H. influenzae* isolates. The microdilution testing was done at the Case Western Reserve University without knowledge of the E-test results. Comparison of the MICs obtained by the E-test and the microdilution method showed that the results of the two methods were comparable (Fig. 1).

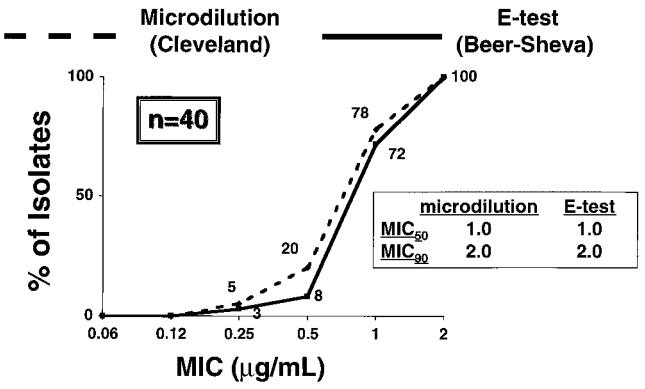

FIG. 1. Comparison of E-test and microdilution MICs for 40 *H. influenzae* isolates. MIC_{50} and MIC_{90}, MICs at which 50 and 90% of isolates are inhibited, respectively.

β-Lactamase production by *H. influenzae* and *M. catarrhalis* was determined by the nitrocefin method (Dryslide nitrocefin; Difco Laboratories, Detroit, Mich.).

The following organisms were considered contaminants: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, coagulase-negative staphylococci, and *Candida* spp.

Clinical scoring. A clinical score was developed to be used for visit 1 (upon enrollment) and visit 2 (on days 4 and 5). This clinical score was described elsewhere (12). In brief, the clinical score was based on the temperature measured at the clinic, report of irritability and ear tugging by the parents, and the appearance and redness of the ear drum observed by the otolaryngologist. The categories of irritability, tugging, redness, and bulging were classified as absent, mild, moderate, or severe. If the eardrum was perforated at the time of the second visit and pus was draining, this was scored by definition as "severe bulging." We did not provide a definition for severity but let the parents and the otolaryngologist freely decide which level of severity to choose. We felt that this approach was appropriate, since on both occasions when the scoring was used, the results of cultures were not known to the parents or the otolaryngologist since specimens for culture were obtained only after the clinician's evaluation. On visit 2, the culture results for specimens obtained during visit 1 were not revealed to the parents or to the otolaryngologist. The maximum score was 15 (when the temperature was >39.0°C and all other categories were judged "severe"), and the minimum score was 0 (when the temperature was <38.0°C and all other categories were judged "absent").

Azithromycin level determinations. Samples selected by convenience drawn on day 4 or 5 were sent to BAS Analytics (a division of Bioanalytical Systems Inc., West Lafayette, Ind.) for azithromycin level determinations.

After receipt at BAS Analytics, aliquots of the middle-ear fluid and serum samples were stored at −70°C until the day of processing. Sample volumes for both serum and middle-ear fluid ranged from 30 to 250 μl. Prior to analysis, sample volumes were adjusted to 1.0 ml; the serum samples were adjusted with blank control serum, and the middle-ear fluid samples were adjusted with a surrogate middle-ear fluid specimen composed of 80% serum and 20% water. After addition of an internal standard (a compound similar in structure to azithromycin, with extraction features similar to those of azithromycin and detected under the same chromatographic conditions as azithromycin), the samples were extracted with methyl *t*-butyl ether. The ether layer was then transferred to a separate tube, evaporated with nitrogen in a heated water bath, and reconstituted with a reconstituting solution (pH 6). Previously prepared calibration standards and quality control samples were extracted with the patient samples. Fifty-microliter injections of the reconstituted sample were made onto a liquid chromatograph. The chromatographic separation took less than 20 min on a zirconium oxide stationary-phase column with a phosphate buffer (pH 10)–acetonitrile mixture as the mobile phase. Detection was achieved with a glassy carbon electrode with an applied potential of 0.85 V. There were no detectable chromatographic interferences in the region of azithromycin or the internal standard.

Azithromycin quantitation was done by calculating the peak height ratio for azithromycin divided by that for the internal standard. A regression line, obtained by linear least-squares analysis of the peak height ratio versus the nominal concentrations of the calibration standards, was used to calculate the concentrations in the patient samples. The quality control samples were used to verify the

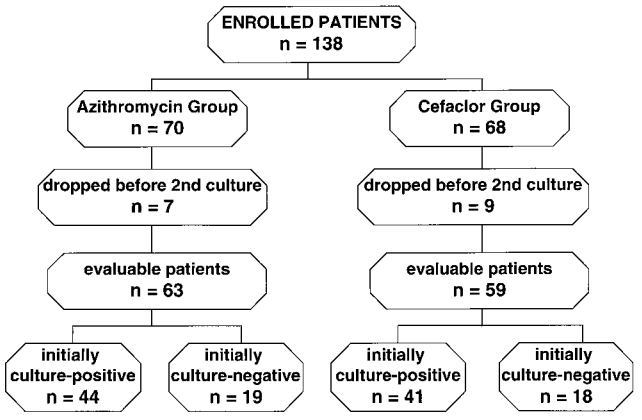

FIG. 2. Flow chart of the study. A total of 138 patients were enrolled, and 122 were evaluable.

accuracy of the calibration line. This accurate and precise assay method had a linear range from 0.0104 to 1.0 μg/ml, based on a 1.0-ml sample volume.

The accuracy of the test ranged from 95.0% at the 0.2-μg/ml level to 97.4% at the 0.05-μg/ml level for serum samples; for middle-ear fluid samples it ranged from 92.4% at the 0.04-μg/ml level to 95% at the 0.4-μg/ml level. Precision in serum ranged from ±0.3% at the 0.5-μg/ml level to ±1.2% at the 0.05-μg/ml level. For middle-ear fluid precision ranged from ±3.0% at the 0.4-μg/ml level to ±5.0% at the 0.04-μg/ml level.

Statistical analysis. The statistical package Epi Info (version 6) was used to test differences in proportions (by the chi-square test or Fisher's exact test, as appropriate). Differences in mean scores were tested by Student's *t* test. A *P* value of <0.05 was considered significant.

RESULTS

A total of 138 patients were enrolled in the study: 70 in the azithromycin group and 68 in the cefaclor group (Fig. 2). Sixteen patients did not have a second tympanocentesis (refused to have a second tympanocentesis or did not return for follow-up, $n = 7$; adverse events, $n = 5$; concomitant disease, $n = 3$; protocol violation, $n = 1$). Thus, the total number of evaluable patients was 122. The demographic and clinical characteristics did not differ between the two groups of patients (Table 1). Of the 122 evaluable patients, 85 were initially culture positive, with a single pathogen isolated from 64 (83%) patients and two or more pathogens isolated from 21 (17%) patients. A total of 55 *H. influenzae* isolates and 44 *S. pneumoniae* isolates were isolated from the specimens retrieved during the initial tympanocentesis during the study (Table 2). Other organisms were *M. catarrhalis* ($n = 5$) and *Streptococcus pyogenes* ($n = 3$). An additional 20 organisms which were not present in the initial culture were isolated during treatment (all except one isolate in the second tympanocentesis): *H. influenzae*, $n = 9$; *S. pneumoniae*, $n = 7$, and *M. catarrhalis*, $n = 4$. Thus, during the study a total of 127 organisms were isolated from the 122 evaluable patients: 64 *H. influenzae*, 51 *S. pneumoniae*, 9 *M. catarrhalis*, and 3 *S. pyogenes* isolates.

The distribution of the MICs of azithromycin and cefaclor for all 64 *H. influenzae* isolates and penicillin, azithromycin, and cefaclor for 48 of the 51 *S. pneumoniae* isolates are shown in Fig. 3. Among the isolates *S. pneumoniae* exhibited a clear bimodal distribution in susceptibility to azithromycin: 81% of strains were susceptible, with azithromycin MICs being very low (≤0.064 μg/ml) for 73% of the strains. Nineteen percent of the *S. pneumoniae* isolates were resistant to azithromycin; all had high-level resistance (azithromycin MICs, ≥32.0 μg/ml). In contrast to the azithromycin susceptibility patterns of the *S. pneumoniae* isolates, the patterns of susceptibility to penicillin and cefaclor showed wider distributions and MICs increased gradually. Only 41% of the pneumococcal isolates were susceptible to penicillin; 53% were intermediate to penicillin, and 6% were fully resistant to penicillin.

All *H. influenzae* isolates were susceptible to azithromycin (MICs, ≤4.0 μg/ml); for 40% azithromycin MICs were 1.0 μg/ml, and for 40% MICs were 2.0 μg/ml. For 59% of the isolates cefaclor MICs were ≤1.0 μg/ml, and for 96% of the isolates MICs were ≤2.0 μg/ml. All but one (98%) of the isolates were susceptible to cefaclor (MICs, ≤8.0 μg/ml).

Of the 64 *H. influenzae* isolates, 12 (19%) were β-lactamase producers. All five *M. catarrhalis* isolates were β-lactamase producers.

We determined (i) bacteriologic failure in initially culture-positive patients (defined by persistence of the organism on day 4 or 5 or recurrence of the organism before day 11), (ii) bacteriologic failure in initially culture-negative patients; and (iii) relapses caused by each organism (Table 3). For this pur-

TABLE 1. Demographic and clinical characteristics of the 122 evaluable patient at enrollment

Characteristic	Azithromycin group ($n = 63$)	Cefaclor group ($n = 59$)
Age (mos)		
Mean ± SD	11.1 ± 6.0	12.0 ± 6.5
Median	8	10
% Males	56	68
% of all patients in the group with the following previous no. of AOM episodes		
0	36	34
1–3	35	35
>3	29	31
% of patients with bilateral otitis media	60	56
% of patients who received antibiotics during the previous 14 days	36	34
Clinical score (mean ± SD)	7.7 ± 2.4	8.0 ± 2.3

TABLE 2. The 107 isolates from middle-ear fluid obtained at initial tympanocentesis from 85 of 122 evaluable patients

Isolate from middle-ear fluid	Azithromycin group (63 evaluable patients)	Cefaclor group (59 evaluable patients)
S. pneumoniae	12	13
H. influenzae	23	12
S. pneumoniae + *H. influenzae*	5	12
S. pneumoniae + others[a]	0	1
S. pneumoniae + *H. influenzae* + others[b]	0	1
H. influenzae + others[c]	2	0
Others[d]	2	2
Total organisms	51	56

[a] *S. pyogenes* ($n = 1$).
[b] *M. catarrhalis* ($n = 1$).
[c] *M. catarrhalis* ($n = 2$).
[d] *M. catarrhalis* ($n = 2$) and *S. pyogenes* ($n = 2$).

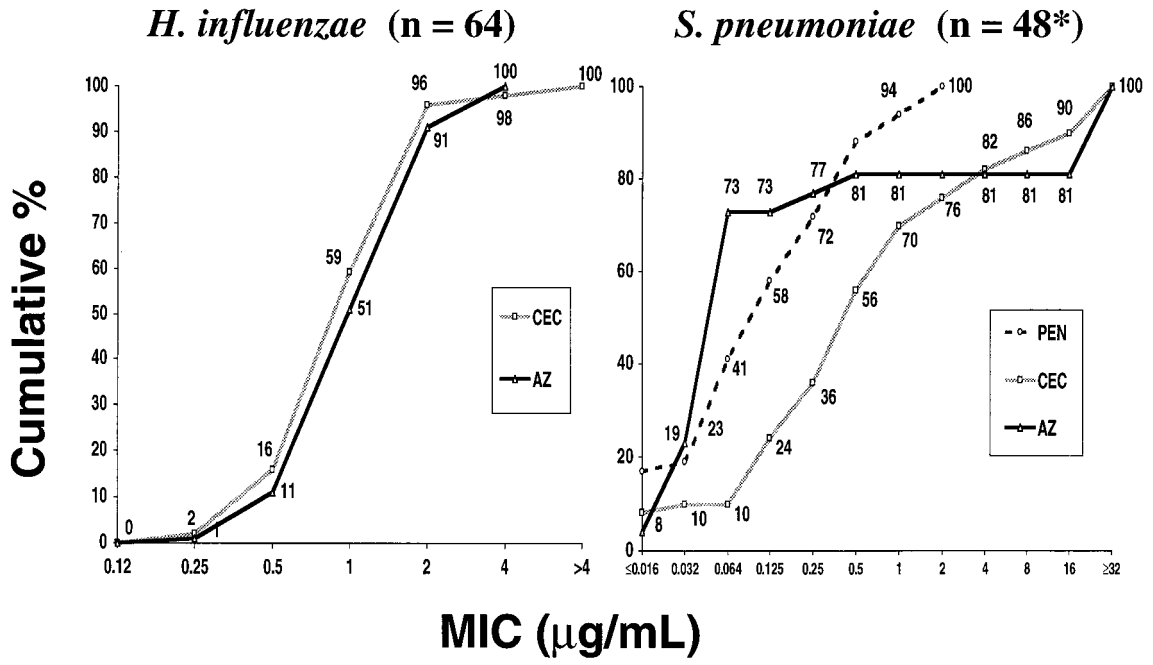

FIG. 3. Cumulative distribution of MICs of various drugs for initial isolates of *S. pneumoniae* ($n = 51$) and *H. influenzae* ($n = 64$). Pen, Penicillin (tested by the E-test); Cec, cefaclor (tested by the E-test); Az, azithromycin (tested by the microdilution method for *S. pneumoniae* and the E-test for *H. influenzae*); *, the azithromycin MICs for three strains were not tested by the microdilution method, and thus, data for these strains were excluded from this comparison.

pose we analyzed the data for each organism, regardless of whether it was isolated alone or concomitantly with other organisms. Thus, a patient could be analyzed more than once, for each organism separately. Patients from whom a pathogen was not present initially but from whom a pathogen was isolated during the second tympanocentesis were listed separately.

Bacteriologic failure was observed in approximately a third of the patients in each group initially positive for *S. pneumoniae* and over half of the patients in each group initially positive for *H. influenzae* (Table 3). No statistically significant differences were noted between the bacteriologic failure rates for the azithromycin and the cefaclor groups with AOM caused by *S. pneumoniae* and *H. influenzae* or in the total rate of bacteriologic failures. In addition, on 20 occasions (occurring in 16% of our patients) a new organism appeared during treatment. Of those, seven were *S. pneumoniae* and nine were *H. influenzae*. This occurred either in initially culture-negative patients or in patients infected with a different organism at enrollment, and all seven new isolates of *S. pneumoniae* were not susceptible to the drug that the patients were receiving. In contrast, all *H. influenzae* isolates that appeared during treatment were susceptible to the respective drugs according to NCCLS cutoff values. Only two cases of true relapse were observed, and both were caused by *H. influenzae* (one in each group) and both were susceptible to the respective drugs according to NCCLS criteria.

Figure 4A presents the bacteriologic failure rates among patients with pneumococcal AOM treated with azithromycin or cefaclor according to the respective drug's MICs. As mentioned above, for azithromycin, the MICs showed a bimodal distribution. Among the isolates from the 18 patients in the azithromycin group, azithromycin MICs were ≤0.06 μg/ml for isolates from 12 patients and bacteriologic failure did not occur in any of them. In contrast, all six patients were infected with strains for which azithromycin MICs were ≥32.0 μg/ml were bacteriologic failures: five strains persisted in initially positive

TABLE 3. Bacteriologic failures in initially culture-positive and initially culture-negative patients

Isolated organism	No. (%) of patients with the following characteristics:					
	Initially culture positive	Bacteriologic failure	Initially culture negative	New organism appeared during treatment	Relapse	All positive
S. pneumoniae						
Azithromycin group	17	5 (29)	46	1 (2)	0	18
Cefaclor group	27	10 (37)	32	6 (19)	0	33
H. influenzae						
Azithromycin group	30	16 (53)	33	5 (15)	1	35
Cefaclor group	25	13 (52)	34	4 (12)	1	29
Others						
Azithromycin group	4	0	59	1 (2)	0	5
Cefaclor group	4	0	55	3 (5)	0	7

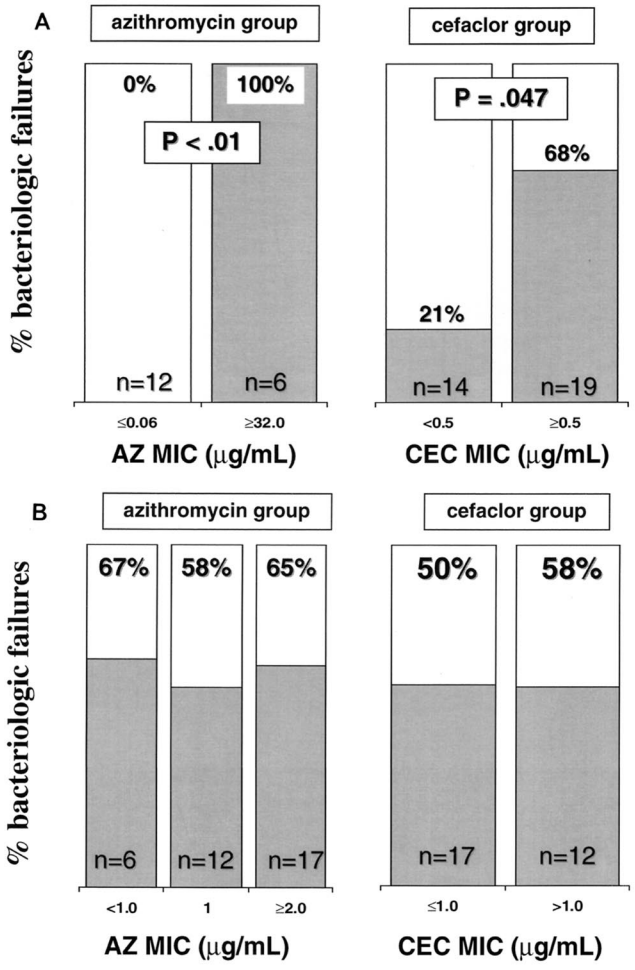

FIG. 4. (A) Bacteriologic failure rate according to MICs for *S. pneumoniae* in patients with AOM treated with azithromycin ($n = 18$) or cefaclor ($n = 33$). (B) Bacteriologic failure rate according to MICs for *H. influenzae* in patients with AOM treated with azithromycin ($n = 35$) or cefaclor ($n = 29$). CEC, cefaclor; AZ, azithromycin. □, cure; ■, failure.

patients and strain one strain appeared during treatment ($P < 0.01$). For the 33 patients in the cefaclor group, the bacteriologic failure rates were compared for those for whose isolates the MIC was <0.5 μg/ml and those for whose isolates the MIC was ≥0.5 μg/ml. The cutoff of 0.5 μg/ml was chosen since in a previous study (14) these values could predict bacteriologic outcome. Bacteriologic failure was observed in 3 of 14 (21%) patients for whose strains the cefaclor MICs were <0.5 μg/ml and 13 of 19 (68%) patients for whose strains cefaclor MICs were ≥0.5 μg/ml ($P = 0.047$).

Figure 4B presents the bacteriologic failure rates among patients infected with *H. influenzae* and treated with azithromycin or cefaclor, according to the respective drug's MICs. A high bacteriologic failure rate was observed both in the azithromycin (17 of 35) and cefaclor (15 of 30) groups, even though all isolates were susceptible (at current breakpoints) to the respective drugs. Among the patients treated with cefaclor, we were not able to compare outcomes for those for whose strains MICs were <0.5 μg/ml and ≥0.5 μg/ml as was done for *S. pneumoniae*, since for only two strains were cefaclor MICs ≤0.5 μg/ml. However, for the patients infected with these two strains, bacteriologic eradication was achieved. No trend of an increase in the bacteriologic failure rate with increasing MICs of the respective drug was observed for patients for whose strain cefaclor MICs were >0.5 μg/ml or for patients treated with azithromycin.

TABLE 4. Serum versus middle-ear fluid azithromycin concentrations in 14 patients from whom both serum and middle-ear fluid specimens were obtained simultaneously on day 4 or 5

Patient no.	Concn (μg/ml)		
	Serum	Left ear	Right ear
54	0.055	ND[a]	3.50
68	0.056	1.68	2.29
69	<0.052	1.88	2.20
75	<0.052	2.00	ND
77	0.324	0.66	ND
78	<0.052	ND	3.96
80	0.067	ND	>4.00
82	<0.104	5.69	ND
95	0.151	ND	6.64
114	<0.052	0.50	0.69
120	<0.104	ND	13.00
131	<0.208	2.46	ND
140	0.011	ND	0.24
142	<0.104	2.68	7.10

[a] ND, not determined.

Azithromycin levels were determined in 28 middle-ear fluid specimens obtained on day 4 or 5 (24 to 48 h after administration of the last dose) from 15 patients, and 14 serum specimens from 15 patients. Simultaneous drug concentrations in serum and middle-ear fluid were obtained from 14 patients on day 4 or 5 (Table 4). The concentrations in serum were consistently low (mean ± standard deviation [SD] = 0.07 ± 0.08 μg/ml). In contrast, the middle-ear fluid concentrations were consistently higher (mean ± SD = 3.51 + 3.21 μg/ml; median = 2.32 μg/ml). The mean ± SD middle-ear fluid azithromycin concentration did not differ significantly between day 4 and 5: they were 2.7 ± 2.1 and 3.9 ± 3.1 μg/ml on days 4 and 5, respectively (range, 0.2 to 13.0 μg/ml).

To determine whether the high bacteriologic failure rate observed among the patients who were infected with *H. influenzae* and who were treated with azithromycin was associated with lower middle-ear fluid azithromycin concentrations, middle-ear fluid azithromycin concentrations were determined for 21 middle-ear fluid specimens from which *H. influenzae* was isolated. No significant difference in middle-ear fluid drug concentrations was found between those from whom *H. influenzae* was eradicated (1.9 ± 2.3 μg/ml) and those from whom the organism was not eradicated (2.4 ± 3.4 μg/ml). The concentration in middle-ear fluid/MIC ratio was above 1 for most patients (10 of 12 in the group with persistent infection or in whom a new organism appeared versus 7 of 9 in the group from which the organism was eradicated.

Of the 63 patients in the azithromycin group, 62 completed at least 10 days of follow-up and thus were clinically evaluable. Of those, 51 (82%) were cured or improved clinically, while 11 (18%) failed clinically. Of the 51 who were clinically well on day 10, 48 (94%) were also seen on day 17 ± 2. Seven of the 48 (15%) relapsed clinically within the 7 days of follow-up. All of the 59 patients in the cefaclor group were monitored at least to day 10. Of these, 50 (85%) were clinically cured or improved and 9 (15%) failed clinically. Of the 50 patients who were clinically well on day 10, 45 were monitored to day 17 ± 2 and 9 (20%) relapsed clinically. No significant differences in outcomes were observed between the cefaclor and azithromycin groups. When clinical outcome was compared to bacteriologic

outcome, 20 of 22 (91%) and 20 of 21 (95%) of those who received azithromycin or cefaclor, respectively, and who had initial positive middle-ear fluid cultures but who had sterile middle-ear fluid by day 4 or 5 had clinical cures. In contrast, only 14 of 21 (67%) and 9 of 16 (56%), respectively, of those still culture positive by day 4 or 5 had clinical cure ($P = 0.002$ for those whose middle-ear fluid became sterile on day 4 or 5 versus those with persistent positive cultures).

Clinical scoring was performed for all patients in the azithromycin ($n = 63$) and cefaclor ($n = 59$) groups on days 1 and 4 or 5 and for 55 and 53 patients in the two groups, respectively on day 10. The mean ± SD scores on day 1, 4 or 5, and 10 were 7.7 ± 2.4, 3.1 ± 2.0, and 1.7 ± 1.5, respectively, for the azithromycin group and 8.0 ± 2.3, 2.9 ± 2.0, and 1.8 ± 1.7, respectively, for the cefaclor group ($P < 0.001$ between day 1 and day 4 or 5 and between day 4 or 5 and day 10 for each group; P was not significant between the azithromycin and cefaclor groups). Thus, a clear improvement in the clinical score was seen during the first 10 days, with no significant difference between the two treatment groups. However, when the score on day 4 or 5 was compared for the 43 patients in whom bacteriologic eradication was observed (in the two groups combined) versus the 37 patients without bacteriologic eradication, a significant difference was observed: 2.9 ± 1.6 versus 3.9 ± 2.3, respectively ($P = 0.02$).

DISCUSSION

Determination of the effect of antibiotics on AOM is a difficult task since in >70% of the patients a spontaneous clinical cure can be observed (35). Therefore, a drug with minimal antibacterial activity, sometimes even a placebo, may appear to be almost as effective as highly efficacious drugs. Since, by definition, the goal of antibiotic treatment in patients with AOM is eradication of the causative organisms from the middle ear, bacteriologic outcome seems to be the most accurate evaluation criterion in studies of the effects of antibiotics against AOM. A clear correlation between bacteriologic outcome on day 4 or 5 and clinical outcome was recently shown by our group (12). Thus, the results of the present investigation on the bacteriologic efficacies of azithromycin and cefaclor against AOM have clear implications for clinical outcome.

The pneumococcal isolates in this study showed the typical distribution in our region in terms of their susceptibilities to penicillin, cefaclor, and azithromycin. Penicillin MICs showed a wide distribution, increasing gradually from 0.016 to 2.0 μg/ml, and cefaclor MICs increased from 0.016 to >16.0 μg/ml. This pattern and distribution are similar to those found in our previous study (14), in which we compared the bacteriologic outcomes achieved with cefaclor to those achieved with cefuroxime axetil. The bimodal distribution of azithromycin MICs for strains for which MICs are low or for strains with high-level macrolide resistance is in accordance with that previously presented in the literature (1).

The bacteriologic response in the patients with pneumococcal AOM in this study clearly reflected the MIC distributions of both cefaclor and azithromycin: the bacteriologic persistence rates on day 4 or 5 were 13 and 53% for cefaclor-treated patients infected with strains for which cefaclor MIC were <0.5 and ≥0.5 μg/ml, respectively, and for 0 and 100% for azithromycin-treated patients infected with strains for which MICs were ≤0.06 and ≥32.0 μg/ml, respectively.

The results of the cefaclor arm of the study are similar to those found in our previous study in which we compared cefaclor to cefuroxime axetil (14) and permit us to use the results for cefaclor to compare the results of the two studies. The findings regarding pneumococcal AOM are important in view of the increasing antibiotic resistance among *S. pneumoniae* isolates. First, on the basis of concentrations achievable in serum and middle-ear fluid concentrations and the MICs of these agents for *S. pneumoniae*, treatment with drugs such as cefaclor, cefixime, and ceftibuten would be predicted to result in bacteriologic failure in patients infected with penicillin-nonsusceptible pneumococci. Thus, the high bacteriologic failure rate of cefaclor in our two recent studies with penicillin-nonsusceptible pneumococci suggests that in areas with a high prevalence of penicillin-nonsusceptible pneumococci, cefaclor (and possibly also cefixime and ceftibuten) may not be useful for the treatment of AOM. Second, the recent increasing rate of resistance of pneumococci to macrolides (which also includes resistance to the azalides) (1, 4, 17, 29, 31), coupled with our failure to eradicate azithromycin-resistant pneumococci from the middle-ear fluid suggests that in regions with a high prevalence of macrolide-resistant pneumococci, drugs belonging to this class should also not be used as empiric treatment for AOM.

Oral cephalosporins and newer macrolides and azalides are often recommended as a second-line antibiotic when initial treatment of AOM with first-line drugs such as amoxicillin or trimethoprim-sulfamethoxazole fail. However, in a recent study (34), we showed that in regions where penicillin-nonsusceptible pneumococci are prevalent, the majority of pneumococci in nonresponsive AOM are not susceptible to penicillin and therefore to cefaclor, thus precluding use of this drug as an empiric second-line drug. Furthermore, it has repeatedly been shown that macrolide-azalide resistance is common among penicillin-nonsusceptible pneumococci (15), which also makes the use of macrolides or azalides as an empiric second line of treatment for AOM problematic, even if the patient was previously treated with a β-lactam drug.

As for the *S. pneumoniae* isolates, the *H. influenzae* isolates had susceptibility patterns (including β-lactamase production) and bacteriologic response rates to cefaclor that were similar to those described in our previous study (14), permitting comparison of data from both studies. In both studies, even though all *H. influenzae* isolates were susceptible to cefaclor according to the definition of NCCLS, a high rate of bacteriologic failure was observed. Furthermore, no significant difference in bacteriologic response rates was found between the isolates for which cefaclor MICs were low (0.38 to 1.0 μg/ml) and those for which MICs were higher (1.5 to 3.0 μg/ml). The high rate of persistence of *H. influenzae* in middle-ear fluid after 3 to 4 days of treatment with cefaclor is consistent with the rate reported in other studies (24, 34, 37): cefaclor had a lower bacteriologic eradication success rate than cefuroxime axetil (14, 27), amoxicillin-clavulanate (34, 41), cefixime (25, 40), and ceftriaxone (27, 32).

The observed frequent failure to eradicate *H. influenzae* from the middle-ear fluid with azithromycin after 3 to 4 days of treatment came as a surprise to us, since all of our *H. influenzae* isolates were susceptible to azithromycin according to current NCCLS definitions (MICs, <4.0 μg/ml; and MICs at which 50% of isolates are inhibited, 1.0 μg/ml). In fact, the observed failure rate in our study is similar to that observed previously for clarithromycin (27) and in the range observed in early studies with placebo (24). This apparent discrepancy was accentuated by the fact that middle-ear fluid azithromycin concentrations 24 to 48 h after administration of the last dose were in most cases still above the MICs for the organisms. Such concentrations above the MIC for a long duration would predict a high bacteriologic cure rate, while the opposite was observed in the present study.

A plausible explanation for these observations may derive from the unique pharmacokinetic and pharmacodynamic properties of azithromycin. Azithromycin concentrations in tissue are far in excess of those observed in serum (17, 45). Azithromycin rapidly reaches high concentrations in human polymorphonuclear cells, with an intracellular concentration-to-extracellular concentration ratio of >226 at 24 h (20). Furthermore, it is suggested that the polymorphonuclear cells and other phagocytes may be important vectors for azithromycin delivery and maintenance at the site of infection (19). Thus, middle-ear fluid produced during AOM in patients treated with azithromycin contains a large number of phagocytes with high intracellular concentrations of azithromycin. To measure middle-ear fluid drug concentrations, the fluid most often is frozen at −20° to −70°C until it is further processed. During the freezing and thawing, these cells are disrupted. Therefore, in most studies, the concentrations of drugs measured in the middle-ear fluid represent a mixture of the intracellular and the extracellular drug concentrations. Using published data, Scaglione (47) projected that the high concentration of azithromycin observed in the middle-ear fluid before removal of the inflammatory cells should become very low after removal of the cells. In fact, the ratio of azithromycin between the cell-free and the cell-containing middle-ear fluid 24 h after drug administration in Scaglione's calculation was strikingly similar to the concentration in serum to the concentration in middle-ear fluid (containing cells) ratio in our study. In view of the information presented above, the role of the newer macrolides and azalides in the treatment of AOM caused by *H. influenzae* must be critically revised.

The clinical cure rates in our patients were 82 and 85% in the cefaclor and azithromycin groups, respectively. Two important points should be made regarding clinical outcome. First, bacteriologic failure rates are usually higher than clinical failure rates (8, 35, 47), and thus it is not surprising to find that despite bacteriologic failure rates of 51 and 42% among the azithromycin and cefaclor groups, respectively, only 18 and 15%, respectively, of the patients had clinical failures. In fact, it was recently shown by our group (12) that only 37% of children who still have culture-positive middle-ear fluid on day 4 or 5 will have a clinical failure.

Second, even though most children were clinically cured, the clinical success rates of both arms of our study seem lower than those in many previously reported studies on azithromycin and cefaclor (2, 6, 30, 36, 43, 44, 46). Our patients were considerably younger than those in most studies (all were <36 months of age; median age, 9 months), while in many published studies the upper age limit was >10 years, with a median of about 4 to 5 years. Higher clinical failure rates among patients younger than 2 years of age compared to those among older children have repeatedly been observed (22, 42). Furthermore, our study group consisted of patients with more severe disease than those usually enrolled in most other studies: 58% had bilateral AOM, and 65% had recurrent AOM (with 30% having more than three previous episodes of AOM). Thus, our lower clinical cure rate compared to those in many other studies reflects the characteristics of the population studied.

In our study, a new organism not present initially was isolated during treatment from 16% of the patients. The two drugs studied in this investigation performed poorly against *H. influenzae* and resistant *S. pneumoniae* strains, and thus, we can speculate that these drugs facilitated nasopharyngeal overgrowth of preexisting *H. influenzae* and nonsusceptible *S. pneumoniae* at the expense of more susceptible organisms. This could facilitate the invasion of the resistant organisms into a diseased, although initially sterile, middle ear.

This study has provided a suitable basis for determining clinically relevant susceptibility breakpoints for the agents evaluated in this study for use against otitis media caused by *S. pneumoniae* and *H. influenzae*. On the basis of pharmacodynamic modeling, Craig (10) and Craig and Andes (11) have suggested that serum β-lactam concentrations need to exceed the MIC for 40 to 50% of the dosing interval to provide high rates of bacteriologic cure. Efficacy with azithromycin, on the other hand, is dependent on the 24-h area under the curve over the MIC ratio exceeding a value of 25, which is equivalent to an average of one time the MIC over a 24-h treatment period (9). These requirements would result in susceptibility breakpoints of 0.5 μg/ml for cefaclor and 0.12 μg/ml for azithromycin. In the case of *H. influenzae*, the MICs of cefaclor and azithromycin for most strains are 1 to 2 μg/ml, and treatment with these agents would be predicted to result in bacteriologic failure as, indeed, they did in this study, and appropriate breakpoints would be at some value below 1.0 μg/ml.

The MICs of cefaclor for *S. pneumoniae* varied much more, with MICs being 0.06 to ≥32 μg/ml, and a clinical breakpoint was apparent at MICs of ≤0.25 μg/ml. This suggests that a breakpoint of 0.25 μg/ml is clinically relevant, and this value is very close to the value of 0.5 μg/ml predicted by Craig (10). As MICs of azithromycin for *S. pneumoniae* are bimodal, determination of a breakpoint is more difficult as strains for which MICs are ≤0.06 μg/ml are clearly susceptible clinically, while MICs were ≥32 μg/ml for resistant strains. However, as bacteriologic failure occurred with azithromycin in patients infected with *H. influenzae*, the clinical breakpoint for azithromycin must be somewhere below the MICs for *H. influenzae* (<1 μg/ml) and above the MICs for macrolide-susceptible *S. pneumoniae* (0.06 μg/ml). As the pharmacodynamically determined breakpoint for azithromycin is 0.12 μg/ml and the current NCCLS breakpoint for azithromycin against *S. pneumoniae* is 0.25 μg/ml, a universal breakpoint for this agent of 0.12 μg/ml appears to be reasonable. The data obtained in this study support changing the current NCCLS breakpoints for cefaclor (8 μg/ml) and azithromycin (4 μg/ml) for *H. influenzae* to 0.25 and 0.12 μg/ml, respectively, for both *H. influenzae* and *S. pneumoniae*.

Two mechanisms of macrolide resistance have been described in *S. pneumoniae* (17, 50). The first is the well-established ribosomal methylase encoded by the *ermAM* gene, which results in high-level resistance to all macrolides, azalides, and lincosamides. The second is a recently described efflux pump, encoded by the *mef* gene, which results in resistance to 14- and 15-membered macrolides as well as to azalides but not to clindamycin or 16-membered macrolides such as rokitamycin and josamycin (50). The latter agents can therefore be used to treat infections caused by organisms with efflux-mediated macrolide resistance. The geographic distribution of the two macrolide resistance mechanisms varies considerably, with efflux-mediated resistance in *S. pneumoniae* being more common in the United States than in Europe or Asia. The fact that all our isolates were highly resistant to azithromycin suggests that the *mef* gene is not frequently present in isolates in our region.

The findings in the present study emphasize the difficulties in choosing an appropriate antibiotic for the treatment of purulent acute AOM in young children in the era of increasing resistance. Nowadays, when choosing antibiotics for the treatment of AOM, one must take into consideration a large number of variables including the patient's age, predisposing factors, previous antibiotic treatment, prevalence of resistant organisms in the community, and the pharmacodynamic properties of the given drugs. However, even if all those factors are

taken into consideration, bacteriologic and clinical failures can still occur.

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