143. Vladrich, P. F., C. Cabellos, R. Pallares, F. Tubau, J. Martinez-Lacasa, J. Liñares, and F. Gudiol. 1996. High doses of cefotaxime in treatment of adult meningitis due to *Streptococcus pneumoniae* with decreased susceptibilities to broad-spectrum cephalosporins. Antimicrob. Agents Chemother. 40:218–220.
adrich, P. F., F. Gudiol, J. Liñares, R. Pallarés, L. Sabaté, G. Rufi, and Artau. 1991. Evaluation of vancomycin for therapy of adult pneumococcal meningitis. Antimicrob. Agents Chemother. 35:2467–2472.
145. Visalli, M. A., M. R. Jacobs, and P. C. Appelbaum. 1996. MIC and time-kill study of activities of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, cefotaxime, imipenem, and vancomycin against nine penicillin-susceptible and -resistant pneumococci. Antimicrob. Agents Chemother. 40:362–366.
146. Visalli, M. A., M. R. Jacobs, and P. C. Appelbaum. 1996. Antipneumococcal activity of Bay 12-8039, a new quinolone compared to 3 other quinolones and 5 β-lactams, abstr. F6. *In* Program and Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
147. Wald, E. R., S. L. Kaplan, E. O. Mason, Jr., D. Sabo, L. Ross, M. Arditi, B. L. Wiedermann, W. Berson, K. S. Kim, R. Yogev, and D. Hoffman. Meningitis Study Group. 1995. Dexamethasone therapy for children with bacterial meningitis. Pediatrics 95:21–28.
148. Welby, P. L., D. S. Keller, J. L. Cromien, P. Tebas, and G. A. Storch. 1994. Resistance to penicillin and non-beta-lactam antibiotics of *Streptococcus pneumoniae* at a children's hospital. Pediatr. Infect. Dis. J. 13:281–287.

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Antimicrobial Resistance of *Streptococcus pneumoniae* Recovered from Outpatients in the United States during the Winter Months of 1994 to 1995: Results of a 30-Center National Surveillance Study

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A total of 1,527 clinically significant outpatient isolates of *Streptococcus pneumoniae* were prospectively collected in 30 different U.S. medical centers between November 1994 and April 1995. Overall, 23.6% of strains were not susceptible to penicillin, with 14.1% intermediate and 9.5% high-level resistant. The frequencies of recovery of intermediate and high-level resistant strains varied considerably between different medical centers and in different geographic areas. In general, intermediate and high-level penicillin resistance was most common with isolates of *S. pneumoniae* recovered from pediatric patients. The in vitro activities of 22 other antimicrobial agents were assessed against this collection of isolates. Ampicillin was consistently 1 twofold dilution less active than penicillin. Amoxicillin and amoxicillin-clavulanate were essentially equivalent to penicillin in activity. The rank order of activity for cephalosporins was cefotaxime = ceftriaxone ≥ cefpodoxime ≥ cefuroxime > cefprozil ≥ cefixime > cefaclor = loracarbef > cefadroxil = cephalexin. The National Committee for Clinical Laboratory Standards [*Performance Standards for Antimicrobial Susceptibility Testing, Sixth Information Supplement (M100-S6)*, 1995] has established MIC breakpoints for resistance (i.e., ≥2 μg/ml) with three cephalosporins versus *S. pneumoniae*, namely, cefotaxime, ceftriaxone, and cefuroxime. The overall percentages of strains resistant to these three antimicrobial agents were 3, 5, and 12, respectively. The overall frequency of resistance was 10% with all three macrolides examined in this study, clarithromycin, erythromycin, and azithromycin. The overall percentages of chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole resistance were 4.3, 7.5, and 18, respectively. The resistance percentages among the cephalosporins, macrolides, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole were consistently higher among penicillin-intermediate strains than among susceptible isolates and even higher still among organisms expressing high-level penicillin resistance. Multiply resistant strains represented 9.1% of the organisms examined in this study. Finally, rifampin resistance was uncommon (i.e., 0.5%), and vancomycin resistance was not detected. The quinopristin-dalfopristin combination was consistently active at concentrations of 0.25 to 4 μg/ml, but rates of resistance could not be determined in the absence of established interpretive criteria for MIC results.

Prior to the early 1990s, penicillin resistance remained uncommon among clinical isolates of *Streptococcus pneumoniae* in the United States despite the emergence of this problem in many other parts of the world (1, 10). In an ongoing national surveillance program conducted by the Centers for Disease Control through the decade of the 1980s, strains of *S. pneumoniae* that were not penicillin susceptible were recovered infrequently, i.e., 3 to 6% (23). Furthermore, among those strains found not to be susceptible to penicillin, nearly all were penicillin intermediate (Pen^i) (i.e., penicillin MICs = 0.1 to 1.0 μg/ml). High-level resistant strains (Pen^r) (i.e., penicillin MICs of ≥2.0 μg/ml) were extremely uncommon. These findings were corroborated by one large independent national surveillance study that characterized 487 isolates from 15 U.S. medical centers in 1987 to 1988 (9). In this study, the percentages of Pen^i and Pen^r strains were found to be 3.8 and 0.2, respectively.

A major increase in the prevalence of penicillin resistance with the pneumococcus evidently took place some time during the early 1990s in the United States, because a national surveillance study performed by Thornsberry and colleagues which characterized 524 isolates from 17 centers during 1991 to 1992 now demonstrated an aggregate percentage of intermediate and high-level resistance of 17.8%, with 15.2% Pen^i and 2.6% Pen^r (24). One year later during 1992 to 1993, Barry and coworkers observed even higher rates of penicillin resistance in a second U.S. national surveillance study (2). Among 799 isolates from 19 U.S. medical centers, the prevalence of Pen^i was 14.9%, and the prevalence of Pen^r was 7.3%.

The mechanism of penicillin resistance is alteration of high-molecular-weight penicillin-binding proteins (PBPs) (11–13). These same PBPs are also important in manifesting the activity of other β-lactams such as the β-lactamase inhibitor combinations, cephalosporins, and carbapenems. As a result, the activity of all of these agents is diminished to at least some extent against pneumococci that are not susceptible to penicillin (2, 3, 6, 9, 12, 22). In general, only cephalosporins with high intrinsic activity against penicillin-susceptible strains (Pen^s) of *S. pneumoniae* (i.e., cefotaxime, ceftriaxone, cefpodoxime, cefuroxime, and perhaps cefprozil) retain sufficient activity against Pen^i strains to be considered of value in treating infections due to such organisms (6, 10). Only cefotaxime and ceftriaxone are thought to be of utility in managing selected infections due to typical pen^r isolates (6, 10). Of great concern are recent reports of clinical isolates of Pen^i *S. pneumoniae* with further alter-

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ations in PBPs that express high-level resistance against cefo- ne and ceftriaxone (4, 7, 20).

ddition to the emergence of β-lactam resistance, isolates *pneumoniae* which are resistant to the macrolides, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX) either alone or in some combination, are now also being recovered increasingly more often from human clinical material in the United States (2, 10). As was the case with β-lactam-resistant pneumococci, multiresistant strains had been recognized as a problem in other parts of the world prior to their emergence in the United States (1, 8, 10).

The intent of the current multicenter national surveillance study was to clearly define the prevalence of antimicrobial resistance among clinical isolates of *S. pneumoniae* in the United States during the winter of 1994 to 1995. Sampling was performed prospectively in such a way as to provide large numbers of community-acquired isolates representative of different geographic areas, different patient populations, and different infectious disease conditions.

MATERIALS AND METHODS

A total of 1,527 isolates of *S. pneumoniae* were collected from 30 different U.S. medical centers between 1 November 1994 and 31 April 1995 (Table 1). In all cases, isolates characterized in this study were recovered from consecutive unique nonhospitalized patients. With the exception of lower respiratory tract specimens, all isolates were obtained from specimens representative of normally sterile body sites (see Table 3). When recovered from lower respiratory tract specimens, only isolates of *S. pneumoniae* judged to be of at least probable clinical significance were included. In contributing study centers, isolates were subcultured onto 5% sheep blood agar plates and incubated overnight at 35 to 37°C in 5 to 7% CO_2. A large amount of colony growth was collected on a rayon swab and immediately immersed in a specially devised, plastic-cap transport tube containing 12 ml of semisolid Aimes transport medium with charcoal (Difco ratories, Detroit, Mich.). Transport tubes were then shipped overnight to coordinating study center, the University of Massachusetts Medical Center, e all additional analyses were performed. The recovery rate from this transport system was 100%.

Isolates were frozen at −70°C in the coordinating study center until further characterization. Following two subcultures, the identity of isolates was confirmed as *S. pneumoniae* by conventional tests and criteria. MICs were determined in Mueller-Hinton broth supplemented with 3% lysed horse blood by the broth microdilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (16, 17). Microdilution trays (final volume of 100 μl per well) were inoculated with ca. 5×10^5 CFU/ml (final concentration) of test organism and incubated for 22 to 24 h at 35°C in ambient air prior to determining MICs. The following 23 antimicrobial agents, obtained as laboratory-grade powders from their respective manufacturers, were tested: penicillin, ampicillin, amoxicillin, amoxicillin-clavulanate (2:1), cefotaxime, ceftriaxone, quinopristin-dalfopristin (30:70), cephalexin, cefadroxil, cefaclor, loracarbef, cefprozil, cefuroxime, cefixime, cefpodoxime, erythromycin, azithromycin, clarithromycin, TMP-SMX (1:19), chloramphenicol, tetracycline, rifampin, and vancomycin. Twelve concentrations of each agent were tested such that off-scale results were obtained only infrequently. *S. pneumoniae* ATCC 49619 and *Haemophilus influenzae* ATCC 49247, ATCC 49766, and ATCC 10211 were used as controls.

Calculations of the percentages of isolates resistant to specific antimicrobial agents were restricted to compounds for which the NCCLS has established MIC interpretive criteria defining the resistant category for *S. pneumoniae* (17). These compounds and criteria were as follows: amoxicillin, amoxicillin-clavulanate, cefotaxime, ceftriaxone, cefuroxime, azithromycin, and vancomycin, all ≥2 μg/ml; clarithromycin and erythromycin, ≥1 μg/ml; chloramphenicol and tetracycline, ≥8 μg/ml; and rifampin and TMP-SMX, ≥4 μg/ml. The NCCLS penicillin breakpoints of 0.1 to 1 μg/ml (intermediate category) and ≥2 μg/ml (resistant category) were also employed.

RESULTS

A total of 1,527 isolates of *S. pneumoniae* were characterized in this study (Table 1). Among these isolates, 216 (14.1%) were Pen^i and 145 (9.5%) were Pen^r. The percentage of strains not ceptible to penicillin, i.e., Pen^i plus Pen^r, varied considerly among contributing study centers from a low of 2.1 at emple University Medical Center in Philadelphia, Pa., to a high of 52.9 at Mt. Sinai Hospital in Miami, Fla. In three centers, the percentages of Pen^i plus Pen^r were ≤10, eight centers had percentages of 11 to 20, nine centers had percentages of 21 to 30, six centers had percentages of 31 to 40, and the remaining four centers had percentages of 41 to 53. Percentages of Pen^i varied between 2.1 and 29.6; Pen^r percentages ranged from 0 to 23.5. In general, the highest percentages of Pen^r were observed in centers with the highest percentages of Pen^i.

Six pediatric hospitals were included in this survey. Pen^i plus Pen^r strains accounted for 30.9% of isolates in these institutions compared with 21.7% in the remaining 24 primarily adult hospitals ($P < 0.005$). This is consistent with the values shown in Tables 2 and 3 where rates of penicillin resistance were sorted by patient age and specimen type. The highest rates of resistance were observed in isolates from patients of ≤5 years of age and from specimens representative of predominantly childhood diseases (i.e., middle ear fluid and sinus aspirates).

Results obtained with 22 other antimicrobial agents are listed in Table 4. Ampicillin appeared to be 1 twofold dilution less active than penicillin for isolates of pneumococci irrespective of penicillin susceptibility category. Amoxicillin and amoxicillin-clavulanate were essentially identical in activity and roughly equivalent to penicillin. Of the cephalosporins tested and compared solely on the basis of MIC values, there emerged a clear rank order of in vitro activity: cefotaxime = ceftriaxone ≥ cefpodoxime ≥ cefuroxime > cefprozil ≥ cefixime > cefaclor = loracarbef > cefadroxil = cephalexin.

By comparing the geometric-mean MICs obtained with a given cephalosporin for Pen^s strains of pneumococci versus the geometric-mean MICs obtained with the same antimicrobial agent for Pen^i and Pen^r strains, the relative influences of the PBP alterations responsible for the two levels of penicillin resistance on cephalosporin activity could be compared (Table 4). The magnitude of increase of cephalosporin MICs as organisms went from Pen^s to Pen^i and from Pen^i to Pen^r varied between 6- and 22-fold with individual antimicrobial agents.

Among the cephalosporins, resistance percentages were calculated only for cefotaxime, ceftriaxone, and cefuroxime, as these are the only agents for which the NCCLS has established interpretive criteria for resistance versus *S. pneumoniae* (17). Overall, 3, 5, and 12% of pneumococcal isolates were resistant to these three antimicrobial agents, respectively. The percentages of resistance among Pen^s, Pen^i, and Pen^r isolates were, respectively, 0, 1, and 32% for cefotaxime; 0, 2, and 50% for ceftriaxone; and 0.4, 36, and 100% for cefuroxime.

Rifampin activity was comparable among Pen^s, Pen^i, and Pen^r strains, with a total of 7 (0.5%) strains resistant to rifampin (i.e., MICs of ≥4 μg/ml) (Table 4). Similarly, no difference in activity was noted with vancomycin and quinopristin-dalfopristin among the three groups of pneumococci, with vancomycin being ca. fourfold more active than the combination. No vancomycin resistance was observed. The frequency of resistance with quinopristin-dalfopristin could not be calculated because of the absence of accepted MIC breakpoints for pneumococci.

The rank order of relative in vitro activities of the three macrolides examined in this study for the pneumococcus was clarithromycin ≥ erythromycin ≥ azithromycin (Table 4) when MIC_{50}s (MICs at which 50% of the isolates are inhibited), MIC_{90}s, and geometric-mean MICs were compared. In terms of overall prevalence of resistance, however, the macrolides appeared equivalent, with 10% of all study isolates found to be resistant to all three agents. Similar observations were made when rates of macrolide resistance were compared with strains in the three penicillin resistance categories. The resistance percentages with all three macrolides were 4 with Pen^s strains, 19 to 20% with Pen^i isolates, and 49% with Pen^r isolates. There

TABLE 1. Recovery of penicillin-resistant *S. pneumoniae* from 30 U.S. medical centers during 1994 and 1995

Medical center	Location	Total no. of isolates	No. (%) of isolates that were penicillin:		
			Susceptible	Intermediate	Resistant
Children's Hospital	Boston, Mass.	36	26 (72.2)	2 (5.6)	8 (22.2)
University of Massachusetts Medical Center	Worcester, Mass.	30	25 (83.3)	4 (13.3)	1 (3.3)
Hartford Hospital	Hartford, Conn.	61	55 (90.2)	3 (4.9)	3 (4.9)
SUNY Medical Center	Syracuse, N.Y.	23	21 (91.3)	2 (8.7)	0 (0.0)
Strong Memorial Hospital	Rochester, N.Y.	58	52 (89.7)	3 (5.2)	3 (5.2)
Columbia-Presbyterian Hospital	New York, N.Y.	64	56 (87.5)	4 (6.3)	4 (6.3)
Temple University Medical Center	Philadelphia, Pa.	47	46 (97.9)	1 (2.1)	0 (0.0)
Geisinger Medical Center	Danville, Pa.	57	45 (78.9)	6 (10.5)	6 (10.5)
National Children's Hospital	Washington, D.C.	60	46 (76.7)	10 (16.5)	4 (6.7)
University of North Carolina Medical Center	Chapel Hill, N.C.	60	40 (66.7)	14 (23.3)	6 (10.0)
DeKalb General Hospital	Decateur, Ga.	61	40 (65.6)	9 (14.8)	12 (19.7)
Mt. Sinai Hospital	Miami, Fla.	17	8 (47.1)	5 (29.4)	4 (23.5)
University of South Alabama Medical Center	Mobile, Ala.	68	54 (79.4)	9 (13.2)	5 (7.4)
Cleveland Clinic	Cleveland, Ohio	42	34 (81.0)	2 (4.8)	6 (14.3)
Henry Ford Hospital	Detroit, Mich.	63	51 (81.0)	11 (17.5)	1 (1.6)
Methodist Hospital	Indianapolis, Ind.	63	50 (79.4)	11 (17.5)	2 (3.2)
Rush Presbyterian Medical Center	Chicago, Ill.	41	26 (63.4)	9 (22.0)	6 (14.6)
Evanston Hospital	Evanston, Ill.	49	42 (85.7)	5 (10.2)	2 (4.1)
Children's Hospital	Milwaukee, Wis.	65	43 (66.2)	13 (20.0)	9 (13.8)
Mayo Clinic	Rochester, Minn.	35	24 (68.6)	7 (20.0)	4 (11.4)
Jewish Hospital	St. Louis, Mo.	56	42 (75.0)	11 (19.6)	3 (5.4)
University of Texas SW Medical Center	Dallas, Tex.	58	45 (77.6)	5 (8.6)	8 (13.8)
Texas Children's Hospital	Houston, Tex.	63	47 (74.6)	6 (9.5)	10 (15.9)
Denver General Hospital	Denver, Colo.	62	53 (85.5)	7 (11.3)	2 (3.2)
Primary Children's Hospital	Salt Lake City, Utah	62	37 (59.7)	15 (24.2)	10 (16.1)
Good Samaritan Hospital	Phoenix, Ariz.	57	34 (59.6)	12 (21.1)	11 (19.3)
Cedar's Sinai Hospital	Los Angeles, Calif.	27	15 (55.6)	8 (29.6)	4 (14.8)
Stanford University Medical Center	Palo Alto, Calif.	44	37 (84.1)	5 (11.4)	2 (4.5)
Kaiser Medical Center	Portland, Oreg.	61	48 (78.7)	9 (14.8)	4 (6.6)
Children's Hospital	Seattle, Wash.	37	24 (64.9)	8 (21.6)	5 (13.5)
Total		1,527	1,166 (76.4)	216 (14.1)	145 (9.5)

was near-complete cross-resistance among the three macrolides, with 97.6% of erythromycin-resistant strains also categorized as being resistant to azithromycin and clarithromycin.

The overall percentages of strains resistant to chloramphenicol, tetracycline, and TMP-SMX were 4.3, 7.5, and 18, respectively (Table 4). As was the case with the cephalosporins and macrolides, the resistance percentages with these agents were always higher with Pen^i isolates than with Pen^s strains and highest with Pen^r organisms. The resistance percentages among Pen^s, Pen^i, and Pen^r isolates were, respectively, 1, 7, and 32 with chloramphenicol, 0.3, 17, and 43 with tetracycline and 6, 40, and 80 with TMP-SMX.

Multiresistant strains are depicted in Table 5. A total of 138 study isolates (9.1%) were noted to be multiresistant when multiresistance was defined as Pen^i (n = 49) or Pen^r (n = 89) plus resistance to at least two of the following compounds: erythromycin, TMP-SMX, chloramphenicol and/or tetracycline. Two patterns of resistance were apparent among the multiresistant strains. Nearly all chloramphenicol-resistant strains were also resistant to either tetracycline (i.e., 61 of 67 [91%]) and/or TMP-SMX (i.e., 54 of 67 [80.6%]). The large majority of strains that were resistant to at least three if not all four of these non-β-lactam agents were also Pen^r, i.e., 58 of 79 (73.4%).

DISCUSSION

It is apparent from the results of this prospective national 30-center surveillance study that penicillin resistance with outpatient isolates of *S. pneumoniae* has emerged as a major problem in the United States. Although the percentages of Pen^i and Pen^r strains varied significant among different medical centers, the overall national percentage was 23.6, with approximately two of every five nonsusceptible strains manifesting Pen^r. As has been reported by others, the highest rates of penicillin resistance were noted with pneumococci from pediatric patients, in particular, those with infections such as otitis media or sinusitis, i.e., conditions often associated with extensive exposure to oral β-lactam antimicrobial agents and the resulting selective pressure (5, 14, 18). Both Pen^i and Pen^r strains were also, however, frequently recovered from adults in the present study, as has been previously reported (19).

Two previous multicenter national surveillance studies have been conducted in the United States since 1990, i.e., the period during which penicillin-resistant pneumococci apparently emerged as a major problem (2, 24). The first study, in 1991 to 1992, found 15.2% Pen^i and 2.6% Pen^r strains (24). The second study, in 1992 to 1993, observed percentages of 13.9 and

TABLE 2. Recovery of penicillin-resistant *S. pneumoniae* from patients grouped by age

Age group (yr)	Total no. of isolates	No. (%) of isolates that were penicillin:		
		Susceptible	Intermediate	Resistant
0–5	501	362 (72.3)	76 (15.2)	63 (12.6)
6–10	53	43 (81.1)	6 (11.3)	4 (7.5)
11–20	52	38 (73.1)	10 (19.2)	4 (7.7)
21–50	356	284 (79.8)	43 (12.1)	29 (8.1)
>50	560	437 (78.0)	79 (14.1)	44 (7.8)

TABLE 3. Recovery of penicillin-resistant *S. pneumoniae* from different specimen types

Specimen	Total no. of isolates	No. (%) of isolates that were penicillin:		
		Susceptible	Intermediate	Resistant
Middle ear fluid	118	68 (57.6)	26 (22.0)	24 (20.3)
Sinus aspirate	52	32 (61.5)	8 (15.4)	12 (23.1)
Conjuctival	105	93 (88.6)	10 (9.5)	2 (1.9)
Sputum	633	475 (75.0)	93 (14.7)	65 (10.3)
Blood	541	437 (80.8)	69 (15.8)	35 (6.5)
Cerebrospinal fluid	31	26 (83.9)	2 (7.7)	3 (9.7)
Other	43	31 (72.1)	8 (25.8)	4 (9.3)

7.3, respectively (2). The results of the current study indicate that the prevalence of Pen^i and Pen^r strains of *S. pneumoniae* continues to increase in the United States. Of perhaps even greater concern is the observation that the relative proportion of nonsusceptible strains composed of Pen^r organisms is also increasing. Currently, ca. 40% of nonsusceptible isolates are Pen^r. Obviously, this has major therapeutic implications insofar as Pen^r organisms are likely to be more refractory to management with penicillin irrespective of the infectious disease condition with which they are associated and are also most resistant to other β-lactam antimicrobial agents.

The results of this study also permit comparisons of the activities of other β-lactam antimicrobial agents versus contemporary pneumococci. Ampicillin was consistently 1 twofold dilution less active than penicillin. Amoxicillin and amoxicillin-clavulanate appeared to be essentially comparable to penicillin in activity. The enhanced activities of these two compounds versus penicillin for Pen^i and Pen^r *S. pneumoniae*, as has been previously described (22), was not observed in this study. Of interest were observations pertaining to resistance percentages with amoxicillin and amoxicillin-clavulanate against Pen^r strains, i.e., 66 and 60%, respectively. These values indicate that a reasonably high percentage of Pen^r isolates of *S. pneumoniae* would be categorized as being something other than resistant to amoxicillin and amoxicillin-clavulanate according to current NCCLS breakpoints (17). Indeed, application of current breakpoints would give these two compounds the appearance of being more active than cefuroxime and nearly as active as cefotaxime and ceftriaxone against Pen^r organisms.

The cephalosporins could be separated into several groups with respect to in vitro activity as expressed by MIC_{50}s, MIC_{90}s, and geometric-mean MICs. Two oral agents, cephalexin and cefadroxil, had limited activity even for Pen^s *S. pneumoniae*. Three other oral cephalosporins, loracarbef, cefaclor, and cefixime, were generally active only against Pen^s strains. One agent, cefprozil, demonstrated at least modest activity versus Pen^i isolates in addition to being uniformly active against Pen^s strains. Two cephalosporins, cefuroxime and cefpodoxime, were, like cefprozil, active against Pen^s strains but demonstrated greater activity than cefprozil against Pen^i isolates. All three of these agents lacked activity against Pen^r isolates. Finally, two parenteral expanded-spectrum cephalosporins, cefotaxime and ceftriaxone, were nearly uniformly active against both the Pen^s and Pen^i strains and demonstrated at least some activity against Pen^r strains.

Resistance to cefotaxime and ceftriaxone has previously been described with *S. pneumoniae* (4, 7, 20). It has typically been observed with Pen^i strains and has been noted to be the

TABLE 4. In vitro activities of 23 antimicrobial agents for 1,527 outpatient isolates of *S. pneumoniae*[a]

Antimicrobial agent	Penicillin-susceptible strains (n = 1,165)				Penicillin-intermediate strains (n = 216)				Penicillin-resistant strains (n = 145)				All strains (n = 1,527)	
	MIC_{50}	MIC_{90}	Range of MICs	Mean MIC[b]	MIC_{50}	MIC_{90}	Range of MICs	Mean MIC	MIC_{50}	MIC_{90}	Range of MICs	Mean MIC	MIC_{50}	MIC_{90}
Penicillin	0.015	0.03	≤0.004–0.06	0.03	0.25	1	0.12–1	0.33	2	4	2–8	2.4	0.015	1
Ampicillin	0.034	0.06	≤0.008–1	0.03	0.5	2	0.06–4	0.51	4	8	2–16	4.0	0.03	2
Amoxicillin	0.015	0.03	<0.004–0.12	0.015	0.25	1	0.03–4	0.25	2	8	1–8	2.0	0.015	1
Amox-clav[c]	0.015	0.03	<0.004–1	0.015	0.25	1	0.03–4	0.25	2	4	1–8	2.0	0.015	1
Cefotaxime	0.015	0.06	<0.004–1	0.015	0.25	1	0.015–4	0.20	1	4	0.5–8	1.42	0.015	1
Ceftriaxone	0.03	0.06	≤0.004–1	0.024	0.25	1	0.008–4	0.25	2	4	0.5–8	1.46	0.03	1
Cefpodoxime	0.03	0.06	≤0.015–4	0.04	0.5	2	0.03–>16	0.58	4	(16)	1–>16	4.75	0.03	2
Cefuroxime	0.03	0.12	≤0.015–8	0.04	0.5	4	0.003–16	0.86	8	16	2–32	7.9	0.03	4
Cefprozil	0.25	0.25	≤0.03–16	0.20	1	8	0.12–32	1.5	16	32	4–64	18	0.25	8
Cefixime	0.25	0.5	≤0.06–32	0.31	4	16	0.12–128	3.5	32	64	8–>128	30	0.25	16
Cefaclor	0.5	1	≤0.06–128	0.71	4	64	0.12–>128	5.0	128	>128	16–>256	109	1	64
Loracarbef	1	2	≤0.06–128	1.0	8	64	0.5–>128	6.3	128	>128	8–>128	83	1	16
Cefadroxil	1	2	≤0.12–128	1.6	8	64	0.5–256	10.3	64	256	16–>256	145	2	64
Cephalexin	2	4	≤0.12–128	2.0	16	128	0.25–>256	14.2	128	>256	32–>256	141	2	128
Rifampin	0.03	0.06	≤0.015–>32	0.03	0.03	0.06	≤0.015–≥32	0.03	0.03	0.06	≤0.015–8	0.03	0.03	0.06
Vancomycin	0.25	0.5	≤0.015–1	0.35	0.25	0.5	0.12–1	0.34	0.25	0.5	0.25–1	0.33	0.25	0.5
Quino-dalfo[d]	1	2	0.12–4	0.97	1	2	0.25–8	1.2	1	2	0.5–4	1.2	1	2
Clarithromycin	≤0.03	0.06	≤0.03–>64	0.03	≤0.03	8	≤0.03–>64	0.07	0.06	>64	≤0.03–>64	2.0	≤0.03	1
Erythromycin	0.06	0.06	≤0.03–>64	0.06	0.06	8	≤0.03–>64	0.10	0.12	>64	≤0.03–>64	2.0	0.06	2
Azithromycin	0.12	0.12	≤0.03–>64	0.11	0.12	8	≤0.03–>64	0.23	0.25	>64	0.06–>64	3.5	0.12	2
Chloramphenicol	2	4	0.12–16	2.6	2	4	1–32	3.0	4	16	2–32	5.1	2	4
Tetracycline	0.12	0.25	≤0.03–64	0.17	0.12	32	0.06–64	0.35	0.25	32	0.06–32	0.89	0.12	0.25
TMP-SMX[e]	0.25	1	≤0.015–16	0.25	1	8	0.03–16	1.1	4	8	0.12–32	3.8	0.25	4

[a] All MICs given are in micrograms per milliliter.
[b] Geometric-mean MIC.
[c] Amox-clav, amoxicillin-clavulanate (2:1). The concentrations listed refer to amoxicillin.
[d] Quino-dalfo, quinopristin-dalfopristin (30:70). The concentrations listed refer to the total of the two streptogramins in the mixture.
[e] TMP-SMX, trimethoprim-sulfamethoxazole (1:19). The concentrations listed refer to trimethoprim.

TABLE 5. Multiply resistant *S. pneumoniae* recovered in a national, multicenter surveillance study which characterized 1,527 outpatient isolates

Resistance pattern (breakpoint [μg/ml])[a]				No. of isolates that were also[b]:			
Erythromycin (≥1)	TMP-SMX (≥4)	Chloramphenicol (≥8)	Tetracycline (≥8)	Pen^r	Pen^i	Pen^s	Total
R	S	S	S	0	9	16	25
S	R	S	S	37	48	55	140
S	S	R	S	1	1	1	3
S	S	S	R	1	5	6	12
R	R	S	S	24	16	20	60
R	S	R	S	1	0	0	1
R	S	S	R	2	10	3	15
S	R	R	S	0	0	0	0
S	R	S	R	0	8	2	10
S	S	R	R	4	0	1	5
R	R	R	S	1	1	0	2
R	R	S	R	15	1	5	21
R	S	R	R	4	0	0	4
S	R	R	R	15	9	0	24
R	R	R	R	23	4	1	28

[a] Organisms are noted as R (resistant) when their MICs were greater than or equal to the listed breakpoint; organisms are noted as S (susceptible) when their MICs were less than the listed breakpoint.

[b] Pen^r, high-level penicillin resistant; Pen^i, intermediate penicillin resistance; Pen^s, penicillin susceptible.

result of specific PBP alterations (7, 15). In the current study, fully 3.2% (cefotaxime) and 5.1% (ceftriaxone) of all study isolates had MICs of ≥2 μg/ml and thus were classified as resistant according to the criteria of the NCCLS (17). There was near-complete cross-resistance between these two agents, to wit, all cefotaxime-resistant isolates were also ceftriaxone resistant. Among the 1.9% of strains that were resistant to ceftriaxone but not resistant to cefotaxime, all but one had cefotaxime MICs of 1.0 μg/ml. The remaining isolate had a cefotaxime MIC of 0.5 μg/ml. It is not clear whether these apparent slight differences in activity between cefotaxime and ceftriaxone represent anything more than subtle in vitro differences in antibacterial effect. It is unlikely that they translate into meaningful differences in clinical utility. Also of interest in this study was the finding that the vast majority of strains resistant to cefotaxime and ceftriaxone were Pen^r, not Pen^i. For instance, among the total of 49 cefotaxime-resistant strains, 2 were Pen^i while 47 were Pen^r. Ceftriaxone resistance was observed with 78 isolates; 5 were Pen^i, and 73 were Pen^r.

One final observation concerning the activity of cephalosporins versus *S. pneumoniae* today in the United States pertains to the extent to which the activity of specific agents decreases for Pen^i and Pen^r strains versus Pen^s isolates. The drop in activity between Pen^s and Pen^i strains was 6- to 22-fold, depending on the individual cephalosporin considered. The same observation could be made of the cephalosporins when activity versus Pen^r strains was compared with the activity against Pen^i strains. Another 6- to 22-fold decrease in activity was observed. This information together with knowledge of the intrinsic activity of a particular cephalosporin versus Pen^s strains permits reasonably accurate predictions of the activity of that agent for Pen^i and Pen^r strains.

The three macrolides examined in this study, clarithromycin, erythromycin, and azithromycin, were characterized by comparable overall percentages of resistance of 10. The overall percentages of chloramphenicol, tetracycline, and TMP-SMX resistance were 4.3, 7.5, and 18, respectively. The percentages of resistance with all four of these antibiotic classes were significantly higher with Pen^r strains than with Pen^i isolates. Resistance was uncommon among Pen^s organisms. Clearly, as has been described previously, resistance to non-β-lactam agents, although mechanistically unrelated to penicillin resistance, occurs most commonly in the United States in strains of *S. pneumoniae* that are also β-lactam resistant (2, 21). Of significance was the observation that 9.1% of isolates of *S. pneumoniae* in this survey were Pen^i (3.2%) or Pen^r (5.9%) and also resistant to at least two of the following four antibiotics or antibiotic classes: macrolides, tetracycline, chloramphenicol, and TMP-SMX. Such isolates are considered multiresistant (10). Indeed, 23 isolates from 18 different centers were Pen^r and resistant to all four of the non-β-lactam agents noted above. Fourteen of these 23 organisms were also resistant to cefotaxime and ceftriaxone (MICs ≥2 μg/ml). Rifampin resistance (i.e., 0.5%) was uncommon.

Among the agents examined in this study, vancomycin was the only compound for which no resistant strains were recognized (MIC_{50}s and MIC_{90}s, 1 and 2 μg/ml, respectively). The quinopristin-dalfopristin combination was consistently active over a narrow concentration range (i.e., 0.25 to 4 μg/ml) with MIC_{50}s, MIC_{90}s, and geometric-mean MICs 4-fold higher than those noted with vancomycin. However, in the absence of accepted interpretive criteria, rates of resistance could not be calculated with this combination.

In conclusion, antimicrobial resistance has clearly emerged as a very serious problem with *S. pneumoniae* in the United States. By analogy based on experiences in other parts of the world, this problem is likely to grow in the future.

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REFERENCES

1. Appelbaum, P. C. 1992. Antimicrobial resistance in *Streptococcus pneumoniae*. Clin. Infect. Dis. 15:77–83.
2. Barry, A. L., M. A. Pfaller, P. C. Fuchs, and R. R. Packer. 1994. In vitro activities of 12 orally administered antimicrobial agents against four species of bacterial respiratory pathogens from U.S. medical centers in 1992 and 1993. Antimicrob. Agents Chemother. 38:2419–2425.
3. Beeley, G. S., J. A. Elliott, M. J. Ostoby, and R. R. Facklam. 1987. Susceptibility of relatively penicillin-resistant *Streptococcus pneumoniae* to newer cephalosporin antibiotics. Diagn. Microbiol. Infect. Dis. 7:21–27.

4. Bradley, J. S., and J. D. Connor. 1991. Ceftriaxone failure in meningitis caused by *Streptococcus pneumoniae* with reduced susceptibility to beta-lactam antibiotics. Pediatr. Infect. Dis. J. 10:871–873.
5. Janoway, N., E. Hawkins, D. Hinds, and E. Mason. 1989. Penicillin susceptibility of *Streptococcus pneumoniae* in a private pediatric hospital in Houston, Texas. Antimicrob. Agents Chemother. 33:1111–1112.
6. Doern, G. V. 1994. *Streptococcus pneumoniae* antibiotic resistance—an emerging problem. Infect. Dis. Clin. Pract. 3:110–112.
7. Figueiredo, A. M. S., J. D. Connor, A. Severia, M. Vitoria, V. Pato, and A. Tomasz. 1992. A pneumococcal clinical isolate with high-level resistance to cefotaxime and ceftriaxone. Antimicrob. Agents Chemother. 36:886–889.
8. Jacobs, M. R., H. J. Koornhof, R. M. Robins-Browne, C. M. Stevenson, Z. A. Vermaak, I. Freiman, G. B. Miller, M. A. Whitcomb, M. Isaacson, J. L. Ward, and R. Austrian. 1978. Emergence of multiply resistant pneumococci. N. Engl. J. Med. 299:735–740.
9. Jorgensen, J. H., G. V. Doern, L. A. Maher, A. W. Howell, and J. S. Redding. 1990. Antimicrobial resistance among respiratory isolates of *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* in the United States. Antimicrob. Agents Chemother. 34:2075–2080.
10. Klugman, K. P. 1990. Pneumococcal resistance to antibiotics. Clin. Microbiol. Rev. 3:171–196.
11. Laible, G., and R. Hakenbeck. 1991. Five independent combinations of mutations can result in low-affinity penicillin-binding protein 2x of *Streptococcus pneumoniae*. J. Bacteriol. 173:6986–6990.
12. Linares, J., T. Alonso, J. L. Perez, J. Ayats, M. A. Dominguez, R. Pallares, and R. Martin. 1992. Decreased susceptibility of penicillin-resistant pneumococci to twenty-four β-lactam antibiotics. J. Antimicrob. Chemother. 30: 279–288.
13. Markiewicz, Z., and A. Tomasz. 1989. Variation in penicillin-binding protein patterns of penicillin-resistant clinical isolates of pneumococci. J. Clin. Microbiol. 27:405–410.
14. Mason, E. O., Jr., S. L. Kaplan, L. B. Lamberth, and J. Tillman. 1992. Increased rate of isolation of penicillin-resistant *Streptococcus pneumoniae* in a children's hospital and in vitro susceptibilities to antibiotics of potential therapeutic use. Antimicrob. Agents Chemother. 36:1703–1707.
15. Munoz, R., C. G. Dowson, M. Daniels, T. J. Coffey, C. Martin, R. Hakenbeck, and B. G. Spratt. 1992. Genetics of resistance to third-generation cephalosporins in clinical isolates of *Streptococcus pneumoniae*. Mol. Microbiol. 6:2461–2465.
16. National Committee for Clinical Laboratory Standards. 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. National Committee for Clinical Laboratory Standards. 1995. Performance standards for antimicrobial susceptibility testing, sixth information supplement (M100-S6). National Committee for Clinical Laboratory Standards, Villanova, Pa.
18. Nava, J. M., F. Bella, J. Garau, J. Lite, M.-A. Morera, C. Marti, D. Fontanals, R. Font, V. Pineda, S. Uriz, F. Doulofeu, A. Calderon, P. Duran, M. Grau, and A. Agudo. 1994. Predictive factors for invasive disease due to penicillin-resistant *Streptococcus pneumoniae*: a population-based study. Clin. Infect. Dis. 19:884–890.
19. Pallares, R., F. Gudiol, J. Linares, J. Ariza, G. Rufi, L. Murgui, J. Dorca, and P. F. Viladrich. 1987. Risk factors and response to antibiotic therapy in adults with bacteremic pneumonia caused by penicillin-resistant pneumococci. New Engl. J. Med. 317:18–22.
20. Sloas, M. M., F. F. Barrett, P. J. Chesney, B. K. English, B. C. Hill, F. C. Tenover, and R. J. Leggiadro. 1992. Cephalosporin treatment failure in penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* meningitis. Pediatr. Infect. Dis. J. 11:662–666.
21. Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum. 1992. Susceptibilities of penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae* to RP 59500, vancomycin, erythromycin, PD 131628, sparfloxacin, temafloxacin, Win 57273, ofloxacin, and ciprofloxacin. Antimicrob. Agents Chemother. 36:856–859.
22. Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum. 1994. In vitro susceptibilities of 185 penicillin-susceptible and -resistant pneumococci to WY-49605 (SUN/SY 5555), a new oral penem, compared with those to penicillin G, amoxicillin, amoxicillin-clavulanate, cefixime, cefaclor, cefpodoxime, cefuroxime, and cefdinir. Antimicrob. Agents Chemother. 38:2902–2904.
23. Spika, J. S., R. R. Facklam, B. D. Plikaytis, M. J. Oxtoby, and the Pneumococcal Surveillance Working Group. 1991. Antimicrobial resistance of *Streptococcus pneumoniae* in the United States, 1979–1987. J. Infect. Dis. 163: 1273–1278.
24. Thornsberry, C., S. D. Brown, C. Yee, S. K. Bouchillon, J. K. Marler, and T. Rich. 1993. Increasing penicillin resistance in *Streptococcus pneumoniae* in the U.S. Suppl. Infect. Med. 93:15–24.

ELSEVIER

Comparative In Vitro Assessment of Sparfloxacin Activity and Spectrum Using Results from Over 14,000 Pathogens Isolated at 190 Medical Centers in the USA

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Sparfloxacin, a new orally administered fluoroquinolone, was tested against 14,182 clinical strains isolated (generally blood stream and respiratory tract cultures) at nearly 200 hospitals in the United States (USA) and Canada. Sparfloxacin activity was compared with 13 other compounds by Etest (AB BIODISK, Solna, Sweden), broth microdilution, or a standardized disk diffusion method. Using the Food and Drug Administration/product package insert MIC breakpoint for sparfloxacin susceptibility (≤0.5 μg/ml), 94% of Streptococcus pneumoniae *(2666 isolates) and 89% of the other streptococci (554 isolates) were susceptible. However, at ≤1 μg/ml (the breakpoint for all nonstreptococcal species) sparfloxacin susceptibility rates increased to 100% and 98%, respectively, for the two groups of streptococci. Only 50% and 65% of pneumococci were susceptible to ciprofloxacin (MIC_{90}, 3 μg/ml) and penicillin (MIC_{90}, 1.5 μg/ml), respectively. Although there were significant differences between regions in the USA in the frequency of penicillin-resistant pneumococcal strains, results indicate that the overall sparfloxacin MIC_{90} was uniformly at 0.5 μg/ml. Nearly all (≥99%)* Haemophilus *species and* Moraxella catarrhalis*, including those harboring β-lactamases, were susceptible to sparfloxacin, ciprofloxacin, and amoxicillin/clavulanic acid. Only cefprozil and macrolides demonstrated lower potency and spectrum against these two species. Sparfloxacin was active against oxacillin-susceptible* Staphylococcus aureus *(96 to 97%),* Klebsiella *spp. (95%), and other tested enteric bacilli (93%). Comparison between broth microdilution MIC and disk diffusion interpretive results for* M. catarrhalis, Staphylococcus aureus, *and the Enterobacteriaceae showed an absolute intermethod categorical agreement of >95% using current sparfloxacin breakpoints, in contrast to those of cefpodoxime for* S. aureus *where a conspicuous discord (98% versus 59%) between methods was discovered. These results demonstrate that sparfloxacin possesses sufficient in vitro activity and spectrum versus pathogens that cause respiratory tract infections (indications), especially strains resistant to other drug classes such as the earlier fluoroquinolones, oral cephalosporins, macrolides, and amoxicillin/clavulanic acid. The sparfloxacin susceptibility breakpoint for streptococci may require modification (≤1 μg/ml) based on the MIC population analysis presented here. A modal MIC (0.38 to 0.5 μg/ml) was observed at the current breakpoint. Regardless, sparfloxacin inhibited 89% (nonpneumococcal* Streptococcus *spp.) to 100% (*Haemophilus *spp.,* M. catarrhalis*) of the isolates tested with a median activity of 97% against indicated species.*

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penicillin-resistant *S. pneumoniae* strains in the United States (USA) have been 25 to 30% overall, with 10 to 15% intermediate susceptibility (MIC, 0.12 to 1 μg/ml) and 7 to 10% of strains with high-level resistance (MIC >1 μg/ml) (Barry et al. 1997; Breiman et al. 1994; Butler et al. 1996; Doern et al. 1996a; Klugman 1996; Klugman et al. 1997). Penicillin resistance exceeds 50% of isolates in some geographic areas and may achieve that rate nationwide by the end of this decade. Furthermore, this emerging β-lactam resistance among strains of *S. pneumoniae* is compounded by increasing resistance to macrolides, tetracyclines, and trimethoprim/sulfamethoxazole as well (Barry et al. 1997; Doern et al. 1996a). Similar β-lactam resistance has been reported for the viridans group streptococci (Alcaide et al. 1995; Doern et al. 1996b; Pfaller and Jones 1997), a group of organisms responsible for high morbidity and mortality in some at-risk patient populations (Bochud et al. 1994).

H. influenzae has a β-lactamase derived from the TEM-1 plasmid-mediated enzyme commonly found in *Escherichia coli* (Jones et al. 1997; Jorgensen et al. 1992). The prevalence of *H. influenzae* strains containing this enzyme has been assessed at 30 to 40% (Jones et al. 1997), and this resistance mechanism compromises the use of amoxicillin and some older oral cephalosporins (cefaclor, cefprozil, loracarbef) that are sensitive to hydrolysis by this enzyme. Nearly all *M. catarrhalis* isolates produce one of two types of β-lactamases (BRO-1 or -2), and amoxicillin has been generally ineffective clinically (Washington et al. 1996). However, most other orally administered β-lactams (oral cephalosporins), macrolides, tetracyclines, trimethoprim/sulfamethoxazole, etc., have remained active.

Other emerging antimicrobial resistances have included the gradual and at times escalated local increases in methicillin (or oxacillin) resistance among staphylococci (Panlilio et al. 1992). Recently, vancomycin-nonsusceptible strains have been reported by Hiramatsu et al. (1997) in Japan, and fluoroquinolones can also be inactive versus oxacillin-resistant isolates (Blumberg et al. 1991). Enterococci have also become more resistant to ampicillin, glycopeptides, and to the synergistic effects of gentamicin or streptomycin (Cormican and Jones 1996). Furthermore, some enteric bacilli have become resistant to extended-spectrum β-lactams such as "third-generation" cephalosporins via the production of extended-spectrum β-lactamases (ESBL) and/or high amounts of Amp C chromosomal-mediated enzymes (Jones et al. 1994).

Candidate drugs, particularly with an oral route of administration, are urgently needed to address these resistance problems. The fluoroquinolones appear to be excellent alternatives to the previous overutilization of β-lactams (oral penicillin and cephalosporins) and macrolides (erythromycin, azithromycin, clarithromycin) for community-acquired infections. However, the earliest fluoroquinolone antimicrobials available in the USA (e.g., ciprofloxacin, ofloxacin) had only moderate microbiologic activity against *S. pneumoniae*, other *Streptococcus* spp., and oxacillin-resistant staphylococci (Cohen et al. 1996). These fluoroquinolones now have reduced activity against many of these clinical species. In fact, reports of clinical failure with these agents have been

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sections. All strains were recent clinical isolates (fall 1996 through spring 1997).

S. pneumoniae, *Haemophilus* spp., and viridans group streptococci were tested using Etest (AB BIODISK, Solna, Sweden) on blood-supplemented Mueller–Hinton agar (Bolmstrom et al. 1988; Jorgensen et al. 1992). Other strains were tested using either disk diffusion (Difco Laboratories, Detroit, MI, or BBL Sensi-Disc™, Becton Dickinson, Cockeysville, MD) on Mueller–Hinton agar *or* using broth microdilution trays manufactured by Dade/MicroScan (Sacramento, CA). Susceptibility tests were performed following the methods described by the NCCLS (NCCLS 1997a,b) and the manufacturer's package insert.

The susceptibility testing results were recorded as the μg/ml MIC or zone diameter in mm and then interpreted by NCCLS (1997c) susceptible criteria or published guidelines (Fuchs et al. 1993) as follows: for *S. pneumoniae*, cefpodoxime at ≤2 μg/ml, ciprofloxacin at ≤1 μg/ml, erythromycin at ≤0.25 μg/ml, penicillin at ≤0.06 μg/ml, and sparfloxacin at ≤0.5 μg/ml; for other *Streptococcus* spp., cefpodoxime at ≤2 μg/ml, ciprofloxacin at ≤1 μg/ml, erythromycin at ≤0.25 μg/ml, penicillin at ≤0.12 μg/ml, and sparfloxacin at ≤1 μg/ml; for *Haemophilus*, amoxicillin/clavulanic acid at ≤4/2 μg/ml, cefprozil at ≤8 μg/ml, ciprofloxacin at ≤1 μg/ml, clarithromycin at ≤8 μg/ml, and sparfloxacin at ≤0.25 μg/ml; for all other species, amoxicillin/clavulanic acid at ≤8/4 μg/ml or ≥18 mm, azithromycin at ≤2 μg/ml, cefpodoxime at ≤2 μg/ml or ≥21 mm, cefprozil at ≤8 μg/ml or ≥18 mm, ceftazidime at ≤8 μg/ml or ≥18 mm, ceftriaxone at ≤8 μg/ml or ≥21 mm, cefuroxime at ≤8 μg/ml or ≥18 mm, cephalothin at ≤8 μg/ml or ≥18 mm, ciprofloxacin at ≤1 μg/ml or ≥21 mm, clarithromycin at ≤2 μg/ml, erythromycin at ≤0.5 μg/ml or ≥23 mm, ofloxacin at ≤2 μg/ml or ≥16 mm, and sparfloxacin at ≤1 μg/ml or ≥19 mm.

Only data sets with acceptable quality control results were analyzed (NCCLS 1997c). Quality control monitoring (R.N.J., D.M.J.) was performed at the University of Iowa (Iowa City, IA). The quality control organisms were *S. aureus* ATCC 25923 or 29213 and *Escherichia coli* ATCC 25922. Acceptable data sets were returned to the coordinating center (C.H.B., J.A.D.) in Buffalo, NY, where the data were entered into a Paradox database customized for this investigation.

Antimicrobial Agents

S. pneumoniae and viridans group streptococci were tested against penicillin, cefpodoxime, erythromycin, ciprofloxacin, and sparfloxacin. *Haemophilus* spp. strains were tested against cefprozil, amoxicillin/clavulanic acid, ciprofloxacin, sparfloxacin, and clarithromycin. For sites that opted to perform disk diffusion testing, *S. aureus*, *M. catarrhalis*, *Klebsiella* spp., and Enterobacteriaceae were tested against ceftriaxone, ceftazidime, cefuroxime, cefprozil, cefpodoxime, cephalothin, amoxicillin/clavulanic acid, erythromycin, ciprofloxacin, ofloxacin, and sparfloxacin. Azithromycin and clarithromycin were added to these agents for those participants which used the MicroScan™ broth microdilution trays.

Subcultures of organisms exhibiting the following resistance phenotypes were requested to be submitted to the microbiology monitoring site at the University of Iowa for further characterization: penicillin-, erythromycin-, cefpodoxime-, or sparfloxacin-resistant streptococci; cefprozil-, amoxicillin/clavulanic acid-, ciprofloxacin-, or sparfloxacin-resistant *Haemophilus* spp.; fluoroquinolone-resistant but oxacillin-susceptible *S. aureus*; *M. catarrhalis* resistant to any antimicrobial other than penicillins; "third-generation" cephalosporin- or cefuroxime-resistant *Klebsiella* spp. (usually ESBL phenotypes); and Enterobacteriaceae resistant to any fluoroquinolone. Identification to species and susceptibility testing were repeated, and the database corrected when necessary. The investigation is ongoing and the reprocessing of referred strains continues to the studies conclusion in late 1997.

RESULTS AND DISCUSSION

In Vitro Comparisons of Five Antimicrobials Tested against *Haemophilus* spp

Table 1 lists the in vitro antimicrobial susceptibility testing results from Etest with sparfloxacin and ciprofloxacin (fluoroquinolone peer drugs), amoxicillin/clavulanic acid, cefprozil, and clarithromycin. For the 2149 *H. influenzae* strains, sparfloxacin was most active (MIC_{50}, 0.012 μg/ml) and possesses a spectrum (≥99% susceptible strains) equal to those of ciprofloxacin and amoxicillin/clavulanic acid. The tested oral cephalosporin (cefprozil) and macrolide (clarithromycin) were significantly less active and had reduced spectrums of 63 to 75% using the NCCLS (1997c) breakpoint concentrations. Resistance to ampicillin was predicted by the chromogenic cephalosporin β-lactamase test. A total of 29% of *H. influenzae* strains were positive by this test.

The other *Haemophilus* spp. (usually *Haemophilus parainfluenzae*) tested were generally similar in their susceptibility patterns to that of *H. influenzae* strains for the fluoroquinolones and amoxicillin/clavulanic acid, e.g., spectrums >99 to 100% and MIC_{50} results at <1 μg/ml. Cefprozil was more active against these *Haemophilus* spp. strains than clarithromycin (64% susceptible), but both of these comparison

ciprofloxacin-susceptible compared with 94% for sparfloxacin at ≤0.5 μg/ml and >99% at ≤1 μg/ml, the latter concentration representing the breakpoint used for ciprofloxacin (NCCLS 1997c).

The streptococcal isolates (531 strains) other than the *S. pneumoniae* were slightly less susceptible than pneumococci to sparfloxacin (89% and 98% versus 94% and >99%; Table 1). A marked difference in erythromycin resistance plus intermediate rates compared with the pneumococci (41% versus 21%) was observed among these streptococci, but the β-lactams (penicillin, cefpodoxime) appeared more active. The greatest resistance was found among viridans group streptococci. The most active oral antimicrobials against all 3073 streptococci tested were sparfloxacin and cefpodoxime.

Figure 2 illustrates the distribution of tested fluoroquinolone MIC results for the 2542 *S. pneumoniae* strains. The sparfloxacin MIC mode was found to be at the breakpoint concentration of 0.5 μg/ml (NCCLS 1997c), with nearly all remaining organism MICs at 0.75 or 1 μg/ml. In contrast, the vast majority of ciprofloxacin MIC results for contemporary pneumococcal strains were at its breakpoint (1 μg/ml) or intermediate (2 μg/ml) concentration (NCCLS 1997). For ciprofloxacin, the same breakpoint (NCCLS 1997c) has been applied for all susceptibility testing except *Neisseria gonorrhoeae* (≤0.06 μg/ml). The latter criteria would be used to predict success when patients were treated by the single-dose ciprofloxacin regimen. However, sparfloxacin has a breakpoint to predict susceptibility for many indicated pathogens of ≤1 μg/ml, but for pneumococcal tests that breakpoint concentration was reduced to ≤0.5 μg/ml. This produces sparfloxacin-nonsusceptible results for approximately 5% of strains in the SPAR Project that have MICs of 1 μg/ml. Uniformity between the organism-specific breakpoints used should be considered, especially when the same clinical dosing regimens are utilized. The streptococcal organisms with sparfloxacin MIC values of 0.75 or 1 μg/ml represent the upper limits of a unimodal MIC population distribution of potentially treatable organisms (Allegra et al. 1996; Aubier et al. 1996; Baquero and Canton 1996; Cohen et al. 1996; Gelfanno et al. 1996; Ortqvist et al. 1996).

Streptococci with resistance to ciprofloxacin (MIC, ≥4 μg/ml) were discovered in the SPAR Project (Table 2). A total of 77 representative strains of *S. pneumoniae* of this resistance phenotype were obtained from participant laboratories and retested against an expanded battery of fluoroquinolones (Bolmstrom et al. 1988). The greatest potency and potentially widest spectrum were exhibited by some investigational agents: clinafloxacin (MIC_{50}, ≤0.12 μg/ml); trovafloxacin (MIC_{50} 0.25 μg/ml); and grepafloxacin (MIC_{50}, 0.5 μg/ml). Among the marketed compounds, sparfloxacin (MIC_{50} 0.5 μg/ml) was fourfold more active than levofloxacin against these ciprofloxacin-resistant pneumococci. Thirty-four high-level ciprofloxacin-resistant strains (MIC ≥32 μg/ml) were identified of which nine were also highly resistant to levofloxacin and eight to sparfloxacin. These high-level fluoroquinolone-resistant isolates also had elevated MIC values for the investigational fluoroquinolones (clinafloxacin, grepafloxacin, trovafloxacin) compared with the other ciprofloxacin-resistant pneumococcal strains.

Table 3 shows the MIC results for *S. pneumoniae* strains indexed by their resistance patterns for penicillin, erythromycin, and cefpodoxime. The sparfloxacin MIC (MIC_{90} 0.5 to 0.75 μg/ml) was *not* influenced by resistance to β-lactams or macrolides. Only 34 to 54% of strains with resistance to other drugs were ciprofloxacin-susceptible, and a slight trend toward coresistance was noted when resistance results were encountered for β-lactams and erythromycin ($p > 0.05$). Cefpodoxime susceptibility was significantly reduced by the penicillin resistance, and erythromycin resistance increased as the penicillin MICs increased, as well. Only 40% of penicillin-resistant pneumococci remain susceptible to macrolide drugs.

Cohen et al. (1996) summarized the results for sparfloxacin tests against *S. pneumoniae* through

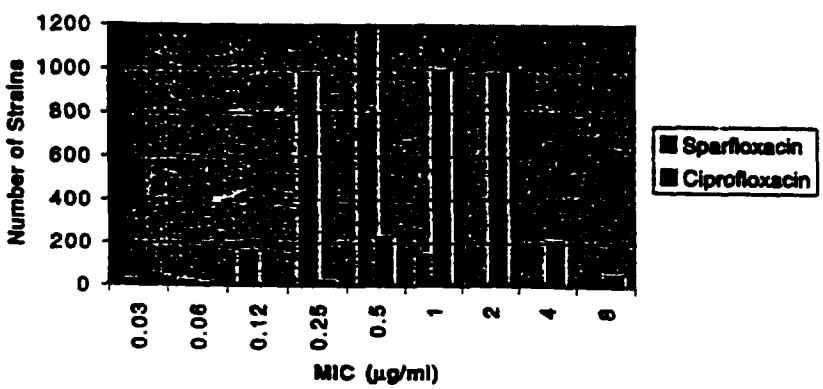

FIGURE 2 MIC population distributions for sparfloxacin and ciprofloxacin tested against 2542 *S. pneumoniae* strains. Vertical broken line indicates the breakpoint for sparfloxacin (≤0.5 μg/ml), and the vertical solid line is the ≤1-μg/ml breakpoint for ciprofloxacin (also proposed for sparfloxacin).

In Vitro Antimicrobial Activity of 13 Drugs Tested against *M. catarrhalis*

Table 4 presents the results for 839 *M. catarrhalis* strains tested against 13 drugs. Only those antimicrobials with potential clinical utility were tested e.g. drugs that were not labile to the β-lactamase produced by more than 90% of these *M. catarrhalis* isolates. Cephalothin (used to predict cephalothin, and oral cephalosporin agents such as cephalexin, cephradine and cephadroxil) and cefprozil demonstrated the narrowest spectrum at 94 to 98% and the least activity among tested β-lactams (MIC_{90}, 4 to 8 μg/ml). This slightly reduced activity was secondary to a partial hydrolysis of these older oral cephems by the β-lactamase enzymes (usually BRO-1) of *M. catarrhalis*. All other orally administered (cefuroxime axetil, cefpodoxime) or parenteral (ceftazidime, ceftriaxone) cephalosporins were effective against >99% of *M. catarrhalis* isolates, as predicted by their greater β-lactamase stability.

The most active agents against *M. catarrhalis* were the fluoroquinolones, sparfloxacin (MIC_{90}, 0.06 μg/ml) and ciprofloxacin (MIC_{90}, 0.06 μg/ml), each inhibiting >99% of strains at or below susceptible breakpoints depending on the susceptibility test method used (NCCLS 1997a,b). The susceptibility testing results produced by the two methods for each drug *did not* vary significantly.

Lastly, eight strains were reported as nonsusceptible to macrolides. Four of these strains were referred to the monitor, and only one was confirmed as having an erythromycin MIC value of >0.5 μg/ml. Although the macrolide-susceptible rates for azithromycin, clarithromycin, and erythromycin are listed at 97 to 98% (Table 4), the actual rate appears to be near 100%, with the *very rare nonsusceptible strains* having only modest increases in the erythromycin MIC (1 to 2 μg/ml). The discovery of this *M. catarrhalis* strain appears to confirm the original observation of Brown et al. (1989) of macrolide-resistant isolates nearly a decade ago, but no trend toward greater occurrence. Documented cases of *M. catarrhalis* strains resistant to fluoroquinolones (Cunliffe et al. 1995), β-lactamase inhibitor combinations, and newer β-lactamase stable cephalosporins have been equally rare or generally unproved.

Activity of 13 Antimicrobials Tested against Oxacillin-Susceptible *S. aureus*

Table 5 lists the in vitro testing results (disk diffusion, MICs) for sparfloxacin compared with 12 other antimicrobial agents. A total of 2556 *S. aureus* strains were tested that were locally determined to be susceptible to oxacillin. Sparfloxacin was the most active drug tested (MIC_{90} 0.12 μg/ml) against these isolates

TABLE 4 In Vitro Testing Results for 839 *M. catarrhalis* Strains Processed by the Broth Microdilution or Disk Diffusion Method

Antimicrobial Agent[a]	Susceptibility Test Method[b]	No. Tested	MIC (μg/ml) 50%	MIC (μg/ml) 90%	Median Zone (mm)	% susceptible
Sparfloxacin	MIC	436	0.03	0.06	—	100
	DISK	403	—	—	38	>99
Ciprofloxacin	MIC	436	0.03	0.06	—	>99
	DISK	403	—	—	37	>99
Ofloxacin	MIC	436	0.06	0.12	—	100
	DISK	403	—	—	34	>99
Cephalothin[c]	MIC	436	4	8	—	98
	DISK	403	—	—	21	94
Cefprozil	MIC	436	2	4	—	98
	DISK	403	—	—	22	94
Cefuroxime	MIC	436	1	2	—	>99
	DISK	403	—	—	30	100
Cefpodoxime	MIC	436	0.5	1	—	>99
	DISK	403	—	—	27	100
Amoxicillin/clavulanic acid	MIC	436	0.12/0.06	0.25/0.12	—	100
	DISK	403	—	—	38	100
Erythromycin	MIC	436	0.12	0.12	—	97
	DISK	403	—	—	34	98
Azithromycin[d]	MIC	436	0.12	0.25	—	99
Clarithromycin[d]	MIC	436	0.12	0.12	—	98

[a] Ceftriaxone and ceftazidime were also tested and were uniformly active.
[b] MIC = broth microdilution (BMD) in Mueller–Hinton broth.
[c] Used to predict oral cephalosporin susceptibility (cephalexin, cephradine, cephadroxil).
[d] Tested only by the BMD method.

In Vitro Susceptibility of Pneumococci to Trovafloxacin, Penicillin G, and Other Antimicrobial Agents in the Czech Republic and Slovakia

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The in vitro activity of the new naphthyridone trovafloxacin (CP 99,219) was compared with those of penicillin G and six other agents (cefpodoxime, erythromycin, azithromycin, clindamycin, ciprofloxacin, and sparfloxacin) against 316 penicillin-susceptible and -resistant pneumococci isolated in the former Czechoslovakia. Trovafloxacin was very active against strains of *Streptococcus pneumoniae* (MIC50 and MIC90 0.25 μg/ml). Ciprofloxacin was less active (MIC50 1.0 μg/ml, MIC90 2.0 μg/ml), and MICs of sparfloxacin were between those of trovafloxacin and ciprofloxacin (MIC50 and MIC90 both 0.5 μg/ml). MICs of cefpodoxime, erythromycin, azithromycin, and clindamycin were higher for strains intermediately resistant or resistant to penicillin than for penicillin-susceptible strains.

In the Czech and Slovak Republics the mean prevalence of penicillin-resistant pneumococci is approximately 8% (P. Urbášková et al., 6th International Congress of Infectious Diseases, Prague, 1994, Abstract no. PCS 78), but marked differences exist locally, particularly in the Slovak Republic, where the incidence is higher than in the Czech Republic (J. Trupl and H. Hupková, 6th International Congress of Infectious Diseases, Prague, 1994, Abstract no. PCS 76). As is the case elsewhere, penicillin-resistant pneumococci isolated in this region are often resistant to other widely used antimicrobial agents (1–3).

The treatment of non-meningeal infections caused by penicillin-resistant pneumococci with high doses of penicillin or other β-lactam antibiotics may be successful (4, 5). Nevertheless, the continued increase in the number of pneumococci resistant to penicillin and other agents emphasizes a need for other classes of effective antimicrobial agents. In the present study the in vitro activity of trovafloxacin (CP99,219), a new benzonaphthyridone (6), was compared with those of penicillin G and six other agents (cefpodoxime, erythromycin, azithromycin, clindamycin, ciprofloxacin and sparfloxacin) against 316 penicillin-susceptible and -resistant pneumococci isolated in the Czech and Slovak Republics.

Materials and Methods. A total of 316 strains of pneumococci isolated from blood, cerebrospinal fluid, sputum, the ear, the eye, and the nasopharynx in the Czech and Slovak Republics during the period 1993–1994 were examined. Identification of strains and determination of MICs were performed in two centers, one in Prague and the other in Bratislava. Before the beginning of the study, both laboratories were checked by external quality control measures, consisting of examination of 11 strains of *Streptococcus pneumoniae* for which MICs of penicillin varied, organized independently by one of the authors (M.R.J.). The susceptibility of selected strains was cross-checked between the two centers during the study.

Of the 110 strains susceptible to penicillin (MIC < 0.125 μg/ml), 35 originated from Slovakia. One hundred twenty-two strains (36 from Slovakia) were intermediately resistant to penicillin (MIC 0.125–1.0 μg/ml), and 84 strains (40 from Slovakia) were resistant to penicillin (MIC ≥ 2.0 μg/ml). MICs were determined by the agar dilution method (7) with Mueller-Hinton agar (BBL Microbiology Systems, USA) supplemented with 5% sheep blood. Antimicrobial agents were supplied as laboratory powders of known potency from their respective manufacturers. For MIC determinations, suspensions equivalent to that of 0.5 McFarland standard were prepared by suspending growth from blood agar plates in 2 ml of Mueller-Hinton broth (Unipath Oxoid, UK). Suspensions were further diluted 1:10 to obtain a final inoculum of 10^4 cfu/spot. Plates were inoculated by a Steers replicator and incubated overnight in ambient air at 37°C. Standard quality control strains *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Streptococcus pneumoniae* ATCC 49619 (7) were included in each run. MICs were determined as the lowest concentration of each antibiotic that inhibited visible growth.

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vak strains (MIC50 0.125 μg/ml and 0.25 μg/ml, respectively). Penicillin MICs of ≥16 μg/ml have been found repeatedly for strains of *Streptococcus pneumoniae* serotype 14 in Slovakia, along with high resistance to other antibiotics (4, 9). This might also explain the higher MICs of trovafloxacin and sparfloxacin.

In summary, the results of this study indicate that trovafloxacin and sparfloxacin possess high in vitro activity against penicillin-susceptible and -resistant strains isolated in the region of former Czechoslovakia. Clinical studies with both compounds in infections caused by penicillin-susceptible and -resistant pneumococci are required to confirm these results.

Acknowledgment

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References

1. Appelbaum PC: Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. Clinical Infectious Diseases 1992, 15: 77–83.
2. Figueiredo AMS, Austrian R, Urbášková P, Teixeira LA, Tomasz A: Novel penicillin-resistant clones of *Streptococcus pneumoniae* in the Czech Republic and Slovakia. Microbial Drug Resistance 1995, 1: 71–78.
3. Klugman KP: Pneumococcal resistance to antibiotics. Clinical Microbiology Reviews 1990, 3: 171–196.
4. Jacobs MR: Treatment and diagnosis of infections caused by drug-resistant *Streptococcus pneumoniae*. Clinical Infectious Diseases 1992, 15: 119–127.
5. Klugman KP: Management of antibiotic-resistant pneumococcal infections. Journal of Antimicrobial Chemotherapy 1994, 34: 191–193.
6. Pankuch GA, Jacobs MR, Appelbaum PC: Activity of CP 99,219 compared with DU-6859a, ciprofloxacin, ofloxacin, levofloxacin, lomefloxacin, tosufloxacin, sparfloxacin and grepafloxacin against penicillin-susceptible and -resistant pneumococci. Journal of Antimicrobial Chemotherapy 1995, 35: 230–232.
7. National Committee for Clinical Laboratory Standards: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. NCCLS, Villanova, PA, 1993.
8. Appelbaum PC: New prospects for antibacterial agents against multidrug-resistant pneumococci. Microbial Drug Resistance 1995, 1: 43–48.
9. Reichler MR, Rakovsky J, Sobotová A, Sláčiková M, Hlaváčová B, Hill B, Krajčíková L, Tarina P, Facklam RR, Breiman RF: Multiple antimicrobial resistance of pneumococci in children with otitis media, bacteremia, and meningitis in Slovakia. Journal of Infectious Diseases 1995, 171: 1491–1496.

In Vitro Activity of Trovafloxacin in Combination with Ceftazidime, Meropenem, and Amikacin

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The in vitro activity of trovafloxacin alone and in combination with ceftazidime, meropenem, and amikacin was studied by determining the minimal inhibitory concentrations (MICs) for 111 gram-negative and 71 gram-positive bacteria. In addition, the synergy of these combinations against 46 strains of gram-negative and gram-positive organisms was studied by checkerboard titration and time-kill kinetics. Trovafloxacin exhibited excellent in vitro activity against all strains tested. Synergism was observed in 17% of the gram-negative strains and in 32% of the gram-positive strains. No antagonism was observed with any of the combinations tested.

The currently available fluoroquinolones possess broad-spectrum antibacterial activity, but they are only moderately active against certain gram-positive organisms such as streptococci, pneumococci, and enterococci and are inactive against anaerobes such as *Bacteroides fragilis* (1, 2). Development of resistance of staphylococci and *Pseudomonas aeruginosa* during therapy has been reported (3). Combining fluoroquinolones with other antimicrobial agents appears to be a reasonable approach to expand the antibacterial spectrum of the fluoroquinolone to prevent the emergence of resistance, and to gain enhanced activity due to synergistic interaction. Trovafloxacin is a new fluoroquinolone, which, in comparison to ofloxacin and ciprofloxacin, is more active against gram-positive bacteria and anaerobes (1, 2, 4, 5). We investigated in vitro the combination of trovafloxacin with, respectively, ceftazidime, meropenem, and amikacin to assess potential

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In Vitro and In Vivo Antibacterial Activities of CS-834, a New Oral Carbapenem

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CS-834 is a prodrug of the carbapenem R-95867, developed by Sankyo Co., Ltd., Tokyo, Japan. To investigate the possibility that CS-834 may be the first carbapenem usable in an oral dosage form, its in vitro antibacterial activity (as R-95867) and in vivo antibacterial activity were compared with those of cefpodoxime proxetil, cefditoren pivoxil, cefdinir, ofloxacin, imipenem, and amoxicillin. R-95867 had high levels of activity against methicillin-susceptible staphylococci and streptococci, including penicillin-resistant *Streptococcus pneumoniae*, as well as *Neisseria gonorrhoeae*, *Moraxella catarrhalis*, the members of the family *Enterobacteriaceae* (with the exception of *Serratia marcescens*), *Haemophilus influenzae*, and *Bordetella pertussis*; for all these strains, the MICs at which 90% of tested strains are inhibited (MIC_{90}s) were 1.0 μg/ml or less. Against methicillin-resistant staphylococci, enterococci, *Serratia marcescens*, *Brukholderia cepacia*, *Stenotrophomonas maltophilia*, and *Acinetobacter calcoaceticus*, R-95867 showed activity comparable to or slightly less than that of imipenem, with MIC_{90}s ranging from 2 to >128 μg/ml. The in vivo efficacy of oral CS-834 against experimental mouse septicemia caused by gram-positive and gram-negative bacteria was better than that of comparative drugs. In murine respiratory infection models, the efficacy of CS-834 reflected not only its potent in vitro activity but also the high levels present in the lungs.

Parenteral carbapenems such as imipenem-cilastatin, meropenem, and panipenem-betamipron have been developed and commercialized (1, 15). These carbapenems have a remarkably broad spectrum of activity against both gram-positive and gram-negative bacteria, including *Pseudomonas aeruginosa*, anaerobes, mycobacteria, and other microorganisms. The carbapenems' remarkable spectrum of in vitro potency appears to result from their ability to penetrate the outer membrane of gram-negative bacilli, as well as their high affinity for certain penicillin-binding protein targets.

Since currently available carbapenems are parenteral drugs, however, the development of oral carbapenems would be of interest. Admittedly, expanded-spectrum cephalosporins stable toward many beta-lactamases from gram-negative bacteria are available in oral as well as parenteral dosage forms. However, members of the family *Enterobacteriaceae* such as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Escherichia coli* have acquired resistance to expanded-spectrum cephem antibiotics by producing extended-spectrum beta-lactamases (7, 8, 14).

The development of oral carbapenems has accordingly been moving toward human therapeutic application. Specifically, Sankyo Co. has synthesized and developed the first oral carbapenem, CS-834 (a prodrug of R-95867). This agent is chemically identified as (+)-[pivaloyloxymethyl-(4*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-4-methyl-7-oxo-3[[(R)-5-oxopyrrolidin-3-yl]-thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate]. To evaluate this drug's potential, we compared the in vitro and in vivo activities of CS-834 (a prodrug of R-95867) with those of cefpodoxime proxetil (a prodrug of cefpodoxime), cefdinir, cefditoren pivoxil (a prodrug of cefditoren), ofloxacin, and imipenem.

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MATERIALS AND METHODS

Drugs. CS-834, R-95867, cefpodoxime, and cefpodoxime proxetil were supplied by Sankyo Co., Ltd. (Tokyo, Japan). The other antimicrobial agents were provided by the indicated manufacturers, as follows: cefdinir, Fujisawa Pharmaceutical Co., Osaka, Japan; cefditoren and cefditoren pivoxil, Meiji Seika Co. Ltd., Tokyo, Japan; ofloxacin, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan; and imipenem, Banyu Pharmaceutical Co., Tokyo, Japan.

Organisms. The clinical isolates tested were obtained from hospitals in several areas of Japan during 1991 and 1996. All organisms had previously been identified by routine laboratory methods and had been stored at −80°C.

In vitro susceptibility tests. The MICs for nonfastidious organisms were determined by the broth microdilution method in 0.1-ml volumes of cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) (9). For fastidious organisms such as *Streptococcus* spp., *Enterococcus* spp., and *Moraxella catarrhalis*, cation-adjusted Mueller-Hinton broth supplemented with 5% lysed horse blood was used (10). For *Haemophilus influenzae*, cation-adjusted Mueller-Hinton broth was supplemented with 5% lysed horse blood plus 5 mg of yeast extract (Oxoid, Hampshire, England) per ml and 15 μg of NAD (Sigma Chemical Co., St. Louis, Mo.) per ml. For all strains, incubation was for 18 to 24 h at 35°C. Microdilution plates were inoculated with an automatic pin inoculator (MIC-2000; Dynatech Laboratories, Inc., Alexandria, Va.) so that the final inoculum was approximately 5×10^5 cfu/ml.

The MICs for *Bordetella pertussis* and *Neisseria gonorrhoeae* were determined by the agar dilution method with Bordet Gengou medium base (Difco) supplemented with 15% horse blood and 1% glycerol and GC II agar (BBL, Baltimore, Md.) supplemented with 1% hemoglobin and IsoVitaleX (BBL), respectively. The bacterial suspension was diluted with saline to a concentration of approximately 10^6 CFU/ml. A portion (about 5 μl) of the diluent was inoculated with an inoculation apparatus (Microplanter; Sakuma Seisakusho, Tokyo, Japan) onto agar plates containing graded concentrations of the drugs at a final inoculum size of 10^4 CFU/spot. Plates inoculated with *B. pertussis* and *N. gonorrhoeae* were incubated at 35°C for 48 h and in 5% CO_2 at 35°C for 24 h, respectively.

The MIC was defined as the lowest concentration of drug resulting in the complete inhibition of visible growth.

In vivo activity. Four-week-old male Slc/ICR mice (weight, 18 to 20 g; Sankyo Labo Service, Tokyo, Japan) were used in groups of 8 to 10 unless indicated otherwise. Test organisms were cultured overnight at 35°C on brain heart infusion agar (Eiken Chemical Co., Ltd., Tokyo, Japan) or blood agar. The organ-

isms were suspended in saline containing 5% mucin (Difco) for bacteremic infection and in saline for local infection.

(i) Bacteremic infection. Mice were challenged intraperitoneally with a single 0.5-ml portion of bacterial suspension (9.5×10^6 CFU of *Staphylococcus aureus* Smith per mouse, 7.1×10^5 CFU of *E. coli* C11 per mouse, or 9.0×10^3 CFU of *Klebsiella pneumoniae* 3K25 per mouse). These inocula were sufficient to cause 100% mortality in untreated animals, with death occurring between 14 and 72 h after infection. Antimicrobial drugs were administered orally 1 h after infection. A 0.05-ml portion of a bacterial suspension of 4.8×10^5 CFU of *Streptococcus pneumoniae* TUH39 (MIC of penicillin G, 0.016 μg/ml) per mouse was instilled intranasally in ketamine-xylazine-anesthetized mice. These inocula caused 100% mortality in untreated animals at 72 to 116 h after infection. Antimicrobial drugs were administered orally two times a day for 3 days starting 20 h after intranasal instillation. The total number of mice surviving at each dose was recorded on day 7 after infection. The 50% effective dose (ED_{50}) of each drug was calculated by the probit method, and the ED_{50} for the pneumococcal infection was expressed as one dose.

(ii) Local infection. Mice under ketamine-xylazine anesthesia were infected by intranasal inoculation (inoculated volume, 0.05 ml) with *S. pneumoniae* TUM741 (MIC of penicillin G, 1.0 μg/ml; 5.1×10^5 CFU/ml), *H. influenzae* TUM8 (4.2×10^5 CFU/ml), or *H. influenzae* TUM36 (5.0×10^5 CFU/ml) (12, 16). Five-week-old female CBA/J mice (weight, 15 to 17 g; Japanese Charles River, Kanagawa, Japan) were used for pneumococcal infection (16). Oral administration of drugs commenced 2 days after infection and was continued for 3 days, with the drugs being given twice a day (at 12-h intervals) or three times a day (at 6-h intervals) for *H. influenzae* infection and three times a day (at 6-h intervals) or four times a day (at 4-h intervals) for *S. pneumoniae* infection.

Animals were sacrificed 20 h after the last administration of the test drug (in order to minimize the influence of the drug administered), and the infected tissues were dissected and homogenized. The number of viable organisms (number of CFU/pair of lungs) was determined by agar plating. Evaluation of efficacy was based on the proportional reduction of the bacterial counts in the infected tissues of treated animals compared with those in the infected tissues of untreated control animals. The statistical significance of the observed differences was determined by the Bonferroni multiple comparison test.

Pharmacokinetics in mice. One day after infection with *S. pneumoniae* TUH39, groups of three mice each received an oral antibiotic at a single dose of 50 mg/kg of body weight. Samples of heart blood and lung and kidney tissues were obtained 5, 15, 30, 60, 120, 240, and 360 min after drug administration. The lungs and kidneys were slightly washed with saline in order to minimize contamination with blood. The levels of the biologically active form of the drug in serum and tissues were determined by a paper disk method, with *E. coli* NIHJJC-2 as the indicator organism for R-95867 and with *Providencia stuartii* IFO12930 as the indicator organism for cefdinir and cefditoren; the respective indicator organisms were incorporated into the medium (nutrient agar; Eiken).

RESULTS

In vitro antibacterial activity. Table 1 presents the comparative in vitro activities of R-95867 and the other drugs tested against a variety of clinical isolates. R-95867 exhibited potent activity against methicillin-susceptible strains of both *S. aureus* and *Staphylococcus epidermidis*, with the MIC at which 90% of tested strains are inhibited (MIC_{90}) being equal to or less than 0.5 μg/ml. Against these species, R-95867 was more active than cefpodoxime, cefditoren, or ofloxacin, but it was less active than imipenem and had almost the same activity as cefdinir. On the other hand, none of the test drugs showed more than minimal potency against methicillin-resistant staphylococci. For *Streptococcus pyogenes* and *Streptococcus agalactiae*, the MIC_{90}s of R-95867 were 0.016 and 0.063 μg/ml, respectively. These values were superior to those for ofloxacin, comparable to those for cefpodoxime, cefdinir, and cefditoren, and inferior to those for imipenem. The MIC_{90} of R-95867 for penicillin-susceptible *S. pneumoniae* was 0.016 μg/ml, which was comparable to that for imipenem, but R-95867 demonstrated a potency at least eight times as great as those of the other comparative drugs. For penicillin-resistant strains of this organism, the MIC_{90} of R-95867 was 0.5 μg/ml; this value is comparable to those for cefditoren and imipenem but represents a potency four or more times as great as those of the other drugs tested.

Among the tested drugs, only imipenem showed significant activity against *Enterococcus faecalis* while none exhibited appreciable activity against *Enterococcus faecium*. The MIC_{90}s of both R-95867 and cefdinir for *N. gonorrhoeae* were 1 μg/ml, representing the highest activity of any agent tested. Against *M. catarrhalis*, the activity of R-95867 (MIC_{90}, 0.125 μg/ml) proved to be comparable to those of ofloxacin and imipenem and superior to those of the cephems tested.

For *E. coli*, *Citrobacter freundii*, *K. pneumoniae*, *K. oxytoca*, *Proteus vulgaris*, *Proteus mirabilis*, and *Morganella morganii*, the MIC_{90}s of R-95867 ranged from 0.016 to 0.25 μg/ml; these values were generally superior to those for the other agents tested. The MIC_{90}s of R-95867 for *Enterobacter cloacae*, *Serratia marcescens*, and *Providencia rettgeri* were 1 or 2 μg/ml, and these levels of activity were roughly comparable to that of imipenem but greater than those of the comparative agents. R-95867 showed little activity against *P. aeruginosa*, which was also true of the comparative agents other than imipenem and ofloxacin. The agent was moderately active against *Burkholderia cepacia* (MIC_{90}, 8 μg/ml), but all of the *Stenotrophomonas maltophilia* isolates were resistant to R-95867.

The MIC_{90}s of R-95867 for *H. influenzae* and *B. pertussis* were 0.25 and 0.5 μg/ml, respectively, and these were generally superior to those for cefpodoxime, cefdinir, and imipenem but inferior to those for cefditoren and ofloxacin. For *Acinetobacter calcoaceticus* isolates the MIC_{90} of R-95867 was 2 μg/ml. This represented activity higher than those of cefpodoxime, cefdinir, cefditoren, or imipenem but lower than that of ofloxacin.

In vivo efficacy in mice. (i) Bacteremic infection. The protective efficacy of CS-834 against experimental bacteremic infections caused by gram-positive and gram-negative bacteria in mice was compared to the protective efficacies of the reference drugs (Table 2). For infection with *S. aureus* Smith, the ED_{50} of CS-834 was 1.30 mg/kg; for the other drugs tested the ED_{50}s were about 1.6 to 27.5 times larger. These values correspond to the respective MICs, which were 2 to 32 times larger for the comparative agents than for R-95867. For infection with *E. coli* C11, CS-834 was as effective as cefpodoxime proxetil and was more effective than cefdinir, cefditoren pivoxil, and ofloxacin. Against *K. pneumoniae* 3K25 infection, the ED_{50} of CS-834 was 6.42 mg/kg, which was comparable to that of cefpodoxime proxetil and which represented a greater effectiveness than those of the other agents tested. By contrast, the MICs of the tested agents for this organism were quite similar.

(ii) Local infection. Against respiratory tract infections induced by inoculation with penicillin-susceptible *S. pneumoniae* TUH39, CS-834 was a little less effective than amoxicillin but was more effective than cefdinir and cefditoren pivoxil (Table 3). When the infection was induced by inoculation with penicillin-resistant *S. pneumoniae*, the mean number of bacteria recovered from the lungs of untreated mice was 7.51 ± 0.37 log CFU/animal (Table 4). Treatment with CS-834 at a dosage of 20 or 50 mg/kg three times a day for 3 days led to significant reductions ($P < 0.05$) in the numbers of bacteria recovered compared with the numbers of bacteria recovered from untreated animals or animals similarly treated with cefdinir, cefditoren pivoxil, or amoxicillin. However, treatment with 10 mg/kg did not significantly reduce the bacterial numbers in any comparison. The other agent demonstrating significant activity in this model was amoxicillin, which at a dose of 50 mg/kg produced significant reductions ($P < 0.05$) in the number of bacteria recovered compared with the numbers of bacteria recovered from control, cefdinir-treated, and cefditoren pivoxil-treated animals.

Among mice with respiratory infections caused by penicillinase-nonproducing *H. influenzae* TUM8, the number of bacteria in the lungs of untreated mice ranged from 5.09 to 5.5 log CFU/g (Table 5). Treatment with CS-834 or amoxicillin at a

TABLE 1. Comparative in vitro activities of R-95867 and reference drugs against clinical isolates

Organism (no. of strains)	Drug	MIC (μg/ml)*		
		Range	50%	90%
Staphylococcus aureus				
Methicillin-susceptible strains (58)	R-95867	0.032–8	0.125	0.25
	Cefpodoxime	1–8	2	4
	Cefdinir	0.032–16	0.5	0.5
	Cefditoren	0.25–8	0.5	1
	Ofloxacin	0.125–32	0.5	1
	Imipenem	0.008–0.25	0.016	0.032
Methicillin-resistant strains (37)	R-95867	0.5–64	32	64
	Cefpodoxime	1–>128	>128	>128
	Cefdinir	0.125–>128	128	>128
	Cefditoren	1–>128	64	128
	Ofloxacin	0.25–128	8	128
	Imipenem	0.063–64	16	64
Staphylococcus epidermidis				
Methicillin-susceptible strains (51)	R-95867	0.032–8	0.063	0.5
	Cefpodoxime	0.25–16	0.5	8
	Cefdinir	0.032–4	0.063	0.5
	Cefditoren	0.063–4	0.25	2
	Ofloxacin	0.063–128	0.25	8
	Imipenem	≤0.004–0.25	0.016	0.125
Methicillin-resistant strains (23)	R-95867	0.5–>128	8	64
	Cefpodoxime	4–>128	16	>128
	Cefdinir	0.125–>128	4	>128
	Cefditoren	2–>128	4	128
	Ofloxacin	0.125–>128	8	>128
	Imipenem	0.063–>128	0.5	64
Streptococcus pyogenes (45)	R-95867	0.008–0.5	0.016	0.016
	Cefpodoxime	≤0.004–2	0.016	0.016
	Cefdinir	≤0.004–1	0.016	0.016
	Cefditoren	≤0.004–1	0.008	0.016
	Ofloxacin	0.5–2	1	2
	Imipenem	≤0.004–0.125	≤0.004	≤0.004
Streptococcus agalactiae (27)	R-95867	0.063	0.063	0.063
	Cefpodoxime	0.032–0.063	0.032	0.063
	Cefdinir	0.032–0.063	0.063	0.063
	Cefditoren	0.032–0.063	0.032	0.032
	Ofloxacin	1–2	2	2
	Imipenem	0.016–0.032	0.016	0.016
Streptococcus pneumoniae				
Penicillin-susceptible strains (36)	R-95867	0.008–0.032	0.008	0.016
	Cefpodoxime	0.008–1	0.032	0.25
	Cefdinir	0.032–2	0.125	0.5
	Cefditoren	≤0.004–0.25	0.016	0.125
	Ofloxacin	1–4	1	2
	Imipenem	≤0.004–0.016	0.008	0.008
Penicillin-resistant strains (32)	R-95867	0.032–0.5	0.25	0.5
	Cefpodoxime	0.063–16	2	2
	Cefdinir	0.125–16	4	8
	Cefditoren	0.032–1	0.5	0.5
	Ofloxacin	1–2	2	2
	Imipenem	0.016–0.5	0.5	0.25
Enterococcus faecalis (28)	R-95867	2–128	8	32
	Cefpodoxime	1–>128	32	>128
	Cefdinir	0.5–>128	4	128
	Cefditoren	2–>128	32	>128
	Ofloxacin	2–>128	2	64
	Imipenem	0.5–128	1	4

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TABLE 1—*Continued*

Organism (no. of strains)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Enterococcus faecium (30)	R-95867	4->128	128	>128
	Cefpodoxime	128->128	>128	>128
	Cefdinir	16->16	>128	>128
	Cefditoren	16->128	>128	>128
	Ofloxacin	2->128	32	128
	Imipenem	1->128	>128	>128
Neisseria gonorrhoeae (27)[b,c]	R-95867	0.016–1	0.063	1
	Cefpodoxime	≤0.004–4	0.063	4
	Cefdinir	≤0.004–1	0.032	1
	Cefditoren	≤0.004–8	0.063	4
	Ofloxacin	0.008–8	0.25	8
	Imipenem	0.25–4	1	4
Moraxella catarrhalis (42)	R-95867	0.016–0.125	0.063	0.125
	Cefpodoxime	0.063–2	0.5	1
	Cefdinir	0.125–0.5	0.25	0.5
	Cefditoren	0.016–1	0.125	0.5
	Ofloxacin	0.063–0.5	0.125	0.125
	Imipenem	0.008–0.125	0.063	0.125
Escherichia coli (30)	R-95867	0.008–0.016	0.016	0.016
	Cefpodoxime	0.125–8	0.25	0.5
	Cefdinir	0.063–8	0.25	0.5
	Cefditoren	0.063–2	0.25	0.25
	Ofloxacin	0.063–0.125	0.063	0.125
	Imipenem	0.063–0.25	0.125	0.125
Citrobacter freundii (38)	R-95867	0.008–2	0.016	0.125
	Cefpodoxime	0.5->128	2	>128
	Cefdinir	0.125->128	1	128
	Cefditoren	0.125->128	1	64
	Ofloxacin	0.063–128	0.25	4
	Imipenem	0.125–0.5	0.25	0.25
Klebsiella pneumoniae (30)	R-95867	0.016–0.125	0.016	0.032
	Cefpodoxime	0.063–32	0.125	0.125
	Cefdinir	0.063–64	0.125	0.125
	Cefditoren	0.125–8	0.25	0.25
	Ofloxacin	0.063–0.25	0.125	0.125
	Imipenem	0.125–0.5	0.125	0.25
Klebsiella oxytoca (38)	R-95867	0.008–0.032	0.016	0.032
	Cefpodoxime	0.032–0.5	0.125	0.25
	Cefdinir	0.032–1	0.125	0.25
	Cefditoren	0.032–0.5	0.125	0.25
	Ofloxacin	0.063–0.5	0.125	0.125
	Imipenem	0.063–1	0.25	0.5
Enterobacter cloacae (29)	R-95867	0.016–2	0.125	1
	Cefpodoxime	0.125->128	1	64
	Cefdinir	0.063->16	2	>128
	Cefditoren	0.125–128	0.5	64
	Ofloxacin	0.063–16	0.063	0.25
	Imipenem	0.125–1	0.25	0.5
Serratia marcescens (30)	R-95867	0.063–16	0.125	2
	Cefpodoxime	0.5->128	1	16
	Cefdinir	2->128	8	>128
	Cefditoren	0.5->128	1	128
	Ofloxacin	0.125–32	0.25	16
	Imipenem	0.25–2	0.25	0.5

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TABLE 1—*Continued*

Organism (no. of strains)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Proteus vulgaris (42)	R-95867	0.016–0.125	0.063	0.125
	Cefpodoxime	0.032–16	0.25	8
	Cefdinir	0.125–64	16	32
	Cefditoren	0.032–128	0.25	2
	Ofloxacin	0.063–2	0.125	0.25
	Imipenem	0.25–4	1	2
Proteus mirabilis (27)	R-95867	0.032–0.25	0.063	0.125
	Cefpodoxime	0.032–0.125	.063	0.125
	Cefdinir	0.063–0.25	0.125	0.125
	Cefditoren	0.032–0.25	0.125	0.125
	Ofloxacin	0.125–0.5	0.25	0.25
	Imipenem	0.5–8	2	4
Morganella morganii (32)	R-95867	0.016–1	0.125	0.25
	Cefpodoxime	0.125–64	1	32
	Cefdinir	0.25–128	8	16
	Cefditoren	0.063–8	0.5	4
	Ofloxacin	0.063–128	0.063	0.25
	Imipenem	0.125–4	1	2
Providencia rettgeri (21)	R-95867	0.016–2	0.125	1
	Cefpodoxime	≤0.004–8	0.032	4
	Cefdinir	≤0.004–128	0.063	8
	Cefditoren	0.016–64	0.125	16
	Ofloxacin	0.063–>128	0.5	64
	Imipenem	0.25–2	1	2
Pseudomonas aeruginosa (30)	R-95867	16–128	32	64
	Cefpodoxime	>128	>128	>128
	Cefdinir	>128	>128	>128
	Cefditoren	32–>128	128	>128
	Ofloxacin	0.5–>128	2	128
	Imipenem	0.5–16	1	16
Burkholderia cepacia (22)	R-95867	0.5–64	4	8
	Cefpodoxime	16–>128	32	64
	Cefdinir	2–128	32	64
	Cefditoren	16–>128	32	64
	Ofloxacin	0.125–32	8	16
	Imipenem	0.125–32	16	32
Stenotrophomonas maltophilia (32)	R-95867	4–>128	>128	>128
	Cefpodoxime	>128	>128	>128
	Cefdinir	>128	>128	>128
	Cefditoren	32–>128	>128	>128
	Ofloxacin	0.5–16	2	4
	Imipenem	8–>128	>128	>128
Haemophilus influenzae (43)	R-95867	0.032–0.25	0.063	0.25
	Cefpodoxime	0.032–0.5	0.063	0.25
	Cefdinir	0.25–2	0.5	1
	Cefditoren	0.008–0.063	0.016	0.032
	Ofloxacin	0.016–0.063	0.032	0.032
	Imipenem	0.125–1	0.5	1
Bordetella pertussis (51)[c,d]	R-95867	0.25–0.5	0.25	0.5
	Cefpodoxime	8–16	16	16
	Cefdinir	32–128	64	64
	Cefditoren	0.25–0.25	0.25	0.25
	Ofloxacin	0.063–0.125	0.063	0.125
	Imipenem	0.5–2	1	1

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TABLE 1—*Continued*

Organism (no. of strains)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Acinetobacter calcoaceticus (28)	R-95867	0.032–4	0.5	2
	Cefpodoxime	0.5–>128	16	>128
	Cefdinir	0.5–>128	4	>128
	Cefditoren	1–>128	32	>128
	Ofloxacin	0.032–4	0.25	0.5
	Imipenem	0.016–4	0.25	4

[a] The MICs were determined by the broth microdilution method with an inoculum of 10^4 CFU per well.
[b] GC II Agar (BBL) supplemented with 1% hemoglobin and IsoVitaleX (Baltimore Biological Laboratories) was used to test *N. gonorrhoeae* isolates, which were incubated in 5% CO_2 at 35°C for 24 h.
[c] The MICs were determined by the agar dilution method with an inoculum 10^4 CFU per spot.
[d] Bordet Gengou agar (Difco) supplemented with 15% horse blood and 1% glycerol was used, and the mixture was incubated at 35°C for 48 h.

dose of 0.125 mg/kg led to significant reductions ($P < 0.05$) in the numbers of organisms in the lungs compared with the numbers of organisms in the lungs of untreated mice. Moreover, the bacterial numbers in the lungs of cefditoren pivoxil-treated mice were below the detectable limits. Among mice with pulmonary infections caused by penicillinase-producing *H. influenzae* TUM36, treatment with CS-834 led to significant reductions ($P < 0.05$) in the number of organisms recovered from the lungs compared with the numbers of organisms recovered from untreated, cefdinir-treated, and amoxicillin-treated mice. The numbers of bacteria in the lungs of cefditoren pivoxil-treated mice were again below the detectable limits.

Effect of cilastatin on the efficacy of CS-834. The activity of the combination of CS-834 and the dehydropeptidase I (DHP-I) inhibitor cilastatin was sometimes, but not always, modestly superior to that of CS-834 alone (Table 6). For murine bacteremic infections caused by various strains, the ED_{50}s of CS-834 alone were 1.1 to 1.7 times larger than those of CS-834–cilastatin. For respiratory infections caused by penicillin-susceptible *S. pneumoniae* TUH39, the addition of cilastatin reduced the ED_{50} by a factor of 2.3. Among mice with murine pulmonary infections caused by penicillin-resistant *S. pneumoniae* TUM741, the level of reduction of the number of organisms in the lungs following treatment with CS-834–cilastatin at a dose of 20 and 50 mg/kg was the same as that following treatment with CS-834 alone at the corresponding dose. However, in contrast to the lack of effect following treatment with CS-834 alone at a dose of 10 mg/kg, treatment with CS-834–cilastatin led to reductions in the numbers of organisms in the lungs that were significant ($P < 0.05$) compared with the reductions in the lungs of animals that were untreated or treated with CS-834, cefdinir, cefditoren pivoxil, or amoxicillin. Among mice with murine pulmonary infections due to penicillinase-nonproducing *H. influenzae* TUM8 or penicillinase-producing *H. influenzae* TUM36, the efficacies of CS-834–cilastatin were almost the same as those of CS-834 alone.

Effect of drug administration interval on the efficacy of CS-834. Among mice with pulmonary infections due to penicillin-resistant *S. pneumoniae*, the numbers of organisms in the lungs of mice administered CS-834–cilastatin four times a day at 4-h intervals, with each dose being 37.5 mg/kg (total daily dose, 150 mg/kg), were below detectable limits. This efficacy was greater than that seen when the same total daily dose was administered as three doses of 50 mg/kg at 6-h intervals. Treatment with CS-834–cilastatin at a dosage of 7.5 mg/kg four times per day led to significant reductions ($P < 0.05$) in the numbers of organisms in the lungs compared with the numbers in the lungs of untreated mice. Among mice with murine pulmonary infections due to penicillinase-nonproducing *H. influenzae* TUM8, the numbers of organisms in the lungs of mice administered 13.3 mg of CS-834–cilastatin per kg three times a day were below the detectable limits. This efficacy was better than that seen with administration of 20 mg/kg twice a day at 12-h intervals.

Drug levels in plasma and tissues of mice infected with *S. pneumoniae*. The levels of R-95867, R-95867–cilastatin, cefdinir, and cefditoren in the sera and lungs of mice infected with *S. pneumoniae* are presented in Fig. 1, and the calculated pharmacokinetics are presented in Table 7. For plasma, the area under the concentration-time curve (AUC), half-life ($t_{1/2}$), and the maximum concentration (C_{max}) of R-95867 were 32.0 μg · h/ml, 1.3 h, and 41.7 μg/ml, respectively; for the lungs, the AUC was 0.7 μg · h/g and the C_{max} was 0.9 μg/ml. Cilastatin increased the AUC of R-95867 in plasma by a factor of 2.3 and the AUC of R-95867 in the lungs by a factor of 10.7. Similarly, the C_{max} in plasma was increased by a factor 1.4 and that in lungs was increased by a factor of 12.2.

TABLE 2. Protective effects of CS-834 and reference drugs against bacteremic infections in mice

Organism	Challenge dose (CFU/mouse)	Drug	MIC (μg/ml)	ED_{50} (mg/kg [95% confidence limit])
S. aureus Smith	9.5×10^6	CS-834	0.063	1.30 (0.90–1.82)
		Cefpodoxime proxetil	2	17.67 (12.82–24.15)
		Cefdinir	0.125	2.10 (0.81–3.15)
		Cefditoren pivoxil	0.5	35.80 (25.16–59.84)
		Ofloxacin	0.25	10.35 (6.50–17.64)
E. coli C11	7.1×10^3	CS-834	0.032	0.27 (0.18–0.35)
		Cefpodoxime proxetil	1	0.35 (0.29–0.42)
		Cefdinir	0.063	1.32 (1.04–1.65)
		Cefditoren pivoxil	0.25	0.94 (0.42–1.36)
		Ofloxacin	0.25	1.00 (0.70–1.30)
K. pneumoniae 31K25	9.0×10^3	CS-834	0.016	6.42 (4.44–15.92)
		Cefpodoxime proxetil	0.125	7.36 (3.87–15.71)
		Cefdinir	0.125	43.30 (31.92–58.74)
		Cefditoren pivoxil	0.25	16.82 (6.52–25.22)
		Ofloxacin	0.125	38.50 (27.58–53.79)

TABLE 3. Therapeutic efficacies of CS-834 and reference drugs against murine respiratory tract infections with penicillin-susceptible *S. pneumoniae* TUH39[a]

Drug	MIC (μg/ml)	ED50 (mg/kg [95% confidence limit])
CS-834	0.016	1.78 (1.20–2.73)
Cefdinir	0.25	19.79 (13.11–33.40)
Cefditoren pivoxil	0.063	5.34 (3.40–7.49)
Amoxicillin	0.032	1.03 (0.55–1.76)

[a] The drugs were administered orally two times a day for 3 days, and the ED_{50} was expressed as one dose. The challenge dose was 4.8×10^6 CFU/mouse.

DISCUSSION

Many types of antibacterial agents (for example, beta-lactams, macrolides, tetracyclines, and quinolones) are available as orally administered drugs. Nevertheless, it is well known that exposure to these antimicrobial agents has led to various types of drug-resistant organisms. Parenteral carbapenems have been developed because they are highly potent against gram-positive cocci and gram-negative bacilli, including *P. aeruginosa*. The recently introduced carbapenems include imipenem, which is highly resistant to the beta-lactamases produced by bacteria. However, it is not resistant to DHP-I derived from kidney and lung tissues and from the brush border of the alimentary tract (5). Therefore, the agent actually injected is a combination of imipenem and the DHP-I inhibitor cilastatin. On the other hand, meropenem is relatively resistant to human DHP-I and is therefore injected alone, even though it is not resistant to the DHP-I of experimental animals (4, 6). This result indicates that carbapenems' stability to DHP-I differs with the source of the enzyme.

Since orally administered penems such as fropenem and ritipenem acoxil are relatively sensitive to DHP-I (2, 17), there is continuing interest in the development of novel oral carbapenems resistant to this enzyme. On the basis of a comparison of the values of the pharmacokinetic parameters for humans (13) and recovery in the urine of mice by coadministration with cilastatin (11) with those found in the present study, we speculate that the stability of R-95867 to DHP-I may be similar to that of meropenem. As a result, use of this new drug in humans may not require simultaneous administration of a DHP-I inhibitor.

R-95867 proved to be a broad-spectrum antibiotic that was slightly less active than imipenem against gram-positive cocci and more active than imipenem against the members of the family *Enterobacteriaceae*. In addition, R-95867 was more active than imipenem against *H. influenzae* and *B. pertussis*, although it lacked activity against *P. aeruginosa*. The data presented here suggest two major clinical indications for oral carbapenems: One might be for treatment of respiratory and urinary tract infections caused by members of the family *Enterobacteriaceae*. Another might be for treatment of infections due to *S. pneumoniae*; both penicillin-susceptible and penicillin-resistant strains proved to be susceptible to R-95867, although penicillin-resistant strains were less so (8).

Against murine bacteremic or lung infections involving various organisms, the efficacy of CS-834 was generally better than those of the reference drugs. CS-834–cilastatin was in general slightly more effective than CS-834 alone. The present study showed that administration of CS-834–cilastatin three times a day was more effective than administration of the combination

TABLE 4. Therapeutic efficacies of CS-834 and reference drugs against murine respiratory tract infection with penicillin-resistant *S. pneumoniae* TUM741

Drug	MIC (μg/ml)	Dose (mg/kg)	Log CFU/lung (mean ± SE)
Control			7.51 ± 0.37
CS-834	0.5	50	3.13 ± 0.18[a]
		20	3.40 ± 0.52[a]
		10	6.61 ± 0.29
Cefdinir	8	50	7.33 ± 0.20
		20	7.33 ± 0.26
		10	7.84 ± 0.20
Cefditoren pivoxil	0.5	50	6.34 ± 0.38
		20	6.19 ± 0.35
		10	6.99 ± 0.15
Amoxicillin	1	50	4.56 ± 0.37[b]
		20	6.99 ± 0.15
		10	7.78 ± 0.23

[a] $P < 0.05$ versus control mice and mice treated with cefdinir, cefditoren pivoxil, and amoxicillin.
[b] $P < 0.05$ versus control mice and mice treated with cefdinir and cefditoren pivoxil.

TABLE 5. Therapeutic efficacies of CS-834 and reference drugs against murine respiratory tract infections with *H. influenzae*

Drug[a]	*H. influenzae* TUM8 (beta-lactamase nonproducing)		*H. influenzae* TUM36 (beta-lactamase producing)	
	MIC (μg/ml)	Log CFU/g (mean ± SE)	MIC (μg/ml)	Log CFU/g (mean ± SE)
Control		5.19 ± 0.32		6.54 ± 0.32
CS-834	0.125	3.09 ± 0.11[b]	0.063	3.90 ± 0.29[b]
Cefdinir	0.5	4.38 ± 0.32	0.25	6.07 ± 0.07
Cefditoren pivoxil	0.016	UD[c]	0.016	UD
Amoxicillin	1	3.73 ± 0.59[b]	32	97.08 ± 0.45

[a] The drugs were administered orally two times a day for 3 days at a dose of 0.125 mg/kg.
[b] $P < 0.05$ versus control mice and mice treated with cefdinir and amoxicillin.
[c] UD, under the lower limit of detectability.

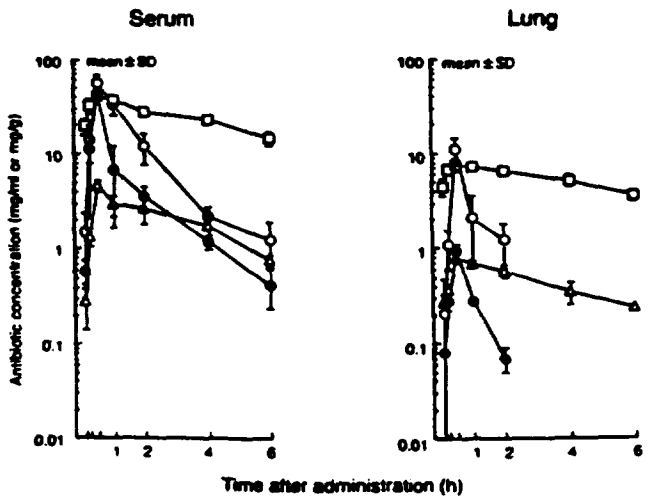

FIG. 1. Levels of R-95867 after oral administration of CS-834 (50 mg/kg) to mice infected with *S. pneumoniae* TUM39 ($n = 3$). ●, R-95867; ○, R-95867–cilastatin; △, cefdinir; □, cefditoren.

TABLE 6. Effect of cilastatin on therapeutic efficacy of CS-834 against bacteremic and respiratory tract infections in mice

Organisms	Challenge dose (CFU/mouse)	Drug	MIC (μg/ml)	Dose (mg/kg)	Therapeutic effect
vens Smith	9.5×10^6	CS-834	0.063		1.30 (0.90–1.82)[a]
		CS-834–cilastatin			0.76 (0.52–1.09)[a]
E. coli C11	7.1×10^3	CS-834	0.032		0.27 (0.18–0.35)[a]
		CS-834–cilastatin			0.18 (0.09–0.25)[a]
K. pneumoniae 3K25	9.0×10^3	CS-834	0.016		6.42 (4.44–15.92)[a]
		CS-834–cilastatin			5.66 (4.14–7.80)[a]
S. pneumoniae TUH39, penicillin susceptible	4.8×10^6	CS-834	0.016		1.78 (1.20–2.73)[a]
		CS-834–cilastatin			0.76 (0.49–1.42)[a]
S. pneumoniae TUM741, penicillin resistant	5.1×10^5	Control			7.51 ± 0.37[b]
		CS-834	0.5	50	3.13 ± 0.18[b,c]
				20	3.40 ± 0.52[b,c]
				10	6.61 ± 0.29[b]
		CS-834–cilastatin		50	3.06 ± 0.21[b,c]
				20	3.76 ± 0.30[b,c]
				10	4.76 ± 0.55[b,d]
H. influenzae TUM8 (beta-lactamase nonproducing)	4.2×10^5	Control			5.19 ± 0.32[d]
		CS-834	0.125	20	3.09 ± 0.11[c,e]
		CS-834–cilastatin		20	3.11 ± 0.12[c,e]
H. influenzae TUM36 (beta-lactamase producing)	1.0×10^5	Control			6.54 ± 0.32[e]
		CS-834	0.063	20	3.90 ± 0.29[c,e]
		CS-834–cilastatin		20	3.60 ± 0.21[c,e]

[a] ED_{50} (milligrams per kilogram [95% confidence limit]).
[b] Log CFU/mouse (mean ± standard error).
[c] $P < 0.05$ versus control mice.
[d] $P < 0.05$ versus control mice and CS-834.
[e] Log CFU/gram (mean ± standard error).

twice a day in murine *H. influenzae* infection models. The same phenomenon was observed in a murine *S. pneumoniae* infection model. This result is in accord with the suggestion that the AUC above the MIC is the pharmacokinetic parameter that best indicates the efficacy of a beta-lactam (3).

Against a murine pulmonary infection caused by *S. pneumoniae*, the efficacy of cefditoren pivoxil proved to be lower than that which would have been predicted from the MIC. A feature of this model is penetration of the infecting organisms into the alveoli (16); thus, poor alveolar penetration by cefditoren pivoxil represents a possible explanation for its unexpectedly poor efficacy. Further studies examining the differences between the in vitro and in vivo activities of this drug should allow a better understanding of this discrepancy.

TABLE 7. Values of pharmacokinetic parameters for CS-834, CS-834–cilastatin, and reference drugs

Specimen	Drug	C_{max} (μg/ml or μg/g)	AUC (μg · h/ml)	$t_{1/2}$ (h)
Plasma	R-95867	41.7	32.0	1.3
	Cefdinir	4.6	9.2	1.5
	Cefditoren	40.6	234.1	3.9
	R-95867-cilastatin	56.8	74.6	1.3
Lung	R-95867	0.9	0.7	ND[a]
	Cefdinir	0.8	3.2	2.4
	Cefditoren	7.7	61.6	5.3
	R-95867-cilastatin	11.0	7.5	ND

[a] ND, not determined.

In conclusion, the studies described here indicate that CS-834 is a promising novel oral carbapenem for the treatment of infections caused not only by gram-negative bacteria but also by gram-positive bacteria, including penicillin-resistant *S. pneumoniae*. The results of clinical trials are awaited with interest.

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REFERENCES

1. Asahi, Y., S. Miyazaki, and K. Yamaguchi. 1995. In vitro and in vivo antibacterial activities of BO-2727, a new carbapenem. Antimicrob. Agents Chemother. 39:1030–1037.
2. Bennett, L., N. Broom, K. Coleman, S. Coulton, P. Edwards, I. Francis, D. Griffin, N. Osborne, and P. Woodall. 1991. 6-(Substituted methylene) penems, potent broad spectrum inhibitors of bacterial beta-lactamase. IV. Kidney stability, serum binding and additional biological evaluation of racemic derivatives. J. Antibiot. 44:338–343.
3. Fantin, B., J. Leggett, S. Ebert, and W. Craig. 1991. Correlation between in vitro and in vivo activity of antimicrobial agents against gram-negative bacilli in a murine infection model. Antimicrob. Agents Chemother. 35:1413–1422.
4. Fukasawa, M., Y. Sumita, E. T. Harabe, T. Tanino, H. Nouda, T. Kohzuki, T. Okuda, H. Matsumura, and M. Sunagawa. 1992. Stability of meropenem and effect of 1β-methyl substitution on its stability in the presence of renal dehydropeptidase I. Antimicrob. Agents Chemother. 36:1577–1579.
5. Gnarpe, H., J. Belsheim, C. Blomqvist, and A. Lundbeck. 1984. Stimulation of granulocyte functions in vitro by imipenem and the renal enzyme inhibitor MK 0791. Antimicrob. Agents Chemother. 25:178–181.
6. Hikida, M., K. Kawashima, M. Yoshida, and S. Mitsuhashi. 1992. Inactivation of new carbapenem antibiotics by dehydropeptidase-I from porcine and human renal cortex. J. Antimicrob. Chemother. 30:129–134.
7. Ishii, Y., A. Ohno, H. Taguchi, S. Imaja, M. Ishiguro, and H. Matsuzawa. 1995. Cloning and sequence of the gene encoding a cefuroxime-hydrolyzing class A beta-lactamase isolated from *Escherichia coli*. Antimicrob. Agents Chemother. 39:2269–2275.

8. Jacobs, C., L. Huang, E. Bartowsky, S. Normark, and J. Park. 1994. Bacterial cell wall recycling provides cytosolic muropeptides as effectors for beta-lactamase induction. EMBO J. 13:4684–4694.
9. Japanese Society for Chemotherapy. 1990. Method for the determination of minimum inhibitory concentration (MIC) of aerobic bacteria by microdilution method. Chemotherapy (Tokyo) 38:102–105.
10. Japanese Society for Chemotherapy. 1993. Method for the determination of minimum inhibitory concentration (MIC) of fastidious bacteria and anaerobic bacteria by microdilution method. Chemotherapy (Tokyo) 41:183–189.
11. Miyauchi, M., R. Endo, M. Hinoeka, H. Yasuda, and I. Kawamoto. 1997. Synthesis and structure-activity relationships of a novel oral carbapenem. CS-834, J. Antibiot. 50:429–439.
12. Miyazaki, S., T. Nunoya, T. Matsumoto, K. Tateda, and K. Yamaguchi. 1997. New murine model of bronchopneumonia due to cell-bound *Haemophilus influenzae*. J. Infect. Dis. 175:205–209.
13. Nakashima, M., K. Umemura, Y. Ikeda, and K. Kondou. 1996. CS-834, a new oral carbapenem. V. Safety and pharmacokinetics in healthy male volunteer. abstr. F119, p. 119. *In* Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
14. Sanders, C. 1987. Chromosomal cephalosporinases responsible for multiple resistance to newer beta-lactam antibiotics. Annu. Rev. Microbiol. 41:573–593.
15. Shimada, J., and Y. Kawahara. 1994. Overview of a new carbapenem, panipenem/betamipron. Drugs Exp. Clin. Res. 20:241–245.
16. Takashima, K., K. Tateda, T. Matsumoto, T. Ito, Y. Iizawa, M. Nakao, and K. Yamaguchi. 1996. Establishment of a model of penicillin-resistant *Streptococcus pneumoniae* pneumonia in healthy CBA/J mice. J. Med. Microbiol. 45:319–322.
17. Tsuji, A., H. Sato, I. Tamai, H. Adachi, T. Nishihara, M. Ishiguro, N. Ohmura, and T. Noguchi. 1990. Physiologically based pharmacokinetics of a new penem, SUN5555, for evaluation of in vivo efficacy. Drug Metab. Dispos. 18:245–252.

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Reprints not available.

1. Starke JR. Nontuberculous mycobacterial infections in children. Adv Pediatr Infect Dis 1992;7:123–59.
2. Marks J, Schivacher H. Infection due to *Mycobacterium xenopi*. Br Med J 1965;1:32–3.
3. Feyen J, Martens M, Mulier JC. Infection of the knee joint with *Mycobacterium xenopi*. Clin Orthop 1983;179:189–90.
4. Sutter AE, Salit IE, Vellend H. The role of *Mycobacterium xenopi* in human disease. Am Rev Respir Dis 1984;129:435–8.
5. Prosser AJ. Spinal infection with *Mycobacterium xenopi*. Tubercle 1986;67:229–32.
6. Jones PG, Schrager MA, Zabransky RJ. Vertebral osteomyelitis with paravertebral abscess due to *M. xenopi*. Clin Microbiol Newlett 1990;12:94–5.
7. Rahman MA, Phongsathorn V, Hughes T, Bielawska C. Spinal infection by *Mycobacterium xenopi*: case report and review. Clin Infect Dis 1994;19:1024–8.
8. Miller WC, Perkins MD, Richardson WJ, Sexton DL. Pott's disease caused by *Mycobacterium xenopi*: case report and review. Clin Infect Dis 1994;19:1024–8.
9. Froideveaux WC, Claudepierre P, Brugières P, Larget-Piet B, Chevalier X. Iatrogenically induced spondilodiskitis due to *Mycobacterium xenopi* in an immunocompetent patient. Clin Infect Dis 1996;22:723–4.
10. Inderlied CB, Kemper CA, Bermudez LEM. The *Myocobacterium avium* complex. Clin Microbiol Rev 1993;6:266–310.

PENETRATION OF CEFPODOXIME INTO MIDDLE EAR FLUID IN PEDIATRIC PATIENTS WITH ACUTE OTITIS MEDIA

The causes of acute otitis media (AOM) are many and varied and result from environmental variables such as supine feeding in young infants, early initiation into group day care, short duration of breast feeding and exposure to cigarette smoke. In addition anatomical factors may be attributable to the increased incidence of AOM in children.

In most instances bacterial invasion of the middle ear is the cause of AOM in children. The most common pathogens associated with the disease are *Streptococcus pneumoniae* accounting for 30 to 50% of infections, *Haemophilus influenzae* (20 to 27%) and *Moraxella catarrhalis* (10 to 15%). Other pathogens that may be responsible for the disease include *Staphylococcus aureus* and *Streptococcus pyogenes* (2 to 5% and 3 to 5%, respectively)[1, 2]

The increasing resistance of the causative pathogens of AOM to antimicrobial agents has become cause for concern. Fifteen to 33% of *Haemophilus influenzae* are beta-lactamase-producing, and ≥75% of *Moraxella catarrhalis* strains produce beta-lactamase.[1] Similarly increased resistance of *S. pneumoniae* to penicillins ranging from intermediately resistant (MICs from 0.1 to 1.0 μg/ml) to highly resistant (MIC >1.0 μg/ml) has also been reported[1–3]

To date the treatment of choice for uncomplicated AOM infections has been amoxicillin. Amoxicillin/clavulanic acid, erythromycin, trimethoprim-sulfamethoxazole combination and the second and third generation cephalosporins have also been used successfully in the treatment of the disease. However, some of these drugs have their limitations. Although amoxicillin is inexpensive, exhibits few side effects and is clinically effective, there is increasing resistance of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* to the drug.[2] From 25 to 50% of *S. pneumoniae* isolates are resistant to trimethoprim-sulfamethoxazole,[2] and *H. influenzae* shows intrinsic resistance to erythromycin.

Orally administered cefpodoxime proxetil is a prodrug that is absorbed and deesterified by the intestinal mucosa to release the antibacterially active third generation cephalosporin, cefpodoxime. Cefpodoxime is resistant to most beta-lactamases of community-acquired pathogens and has a broad spectrum of antibacterial activity encompassing Gram-positive and Gram-negative bacteria.

Cefpodoxime is effective *in vitro* against *H. influenzae* and *M. catarrhalis* including beta-lactamase-producing strains with a minimum inhibitory concentration for 90% of strains (MIC_{90}) ≤1 mg/l. In addition cefpodoxime has an MIC_{90} against penicillin-susceptible strains of *S. pneumoniae* of 0.03 to 0.06 and 1 to 4 mg/l against intermediately penicillin-resistant strains. The MIC_{90} is ≤0.5 mg/l for beta-lactamase-negative and ≤1 mg/l for beta-lactamase-producing strains of *M. catarrhalis*.[4]

Because there are no published data on the penetration of cefpodoxime into middle ear fluid (MEF), this study was undertaken to determine whether cefpodoxime penetrates MEF and to measure the concentration of cefpodoxime in serum and in MEF of children with AOM after a single oral dose administration.

Methods. A small percentage of children who experience multiple recurrent episodes of bilateral AOM require placement of myringotomy tubes.[5] Children with persistent AOM and who as a result of the severity of the infection required myringotomy were selected for inclusion into the study.

Thirty-three samples were taken either unilaterally or bilaterally from twenty-four children ages 5 months to 9 years and diagnosed as having AOM. Myringotomy was performed under general anesthesia (in South Africa, it is current medical practice to perform myringotomy, under general anesthesia, for persistent AOM). Before the surgical procedure parents of the patients were invited to enroll their children in the study. All patients had an intact eardrum before surgery. Patients were excluded if they presented with any of the following: history of hypersensitivity reaction to either cephalosporin or penicillin antibiotics; or ingestion of systemic antimicrobial agents within 48 h before enrollment. The parent(s) or legal guardian of the child was informed as to the nature and purpose of the trial with respect to the product and the procedures involved in the study. After this and before enrollment of the patient into the study, written consent was obtained from the parent(s) or legal guardian of the patient. The protocol was submitted to the independent ethics committee of the Medical Association of South Africa for review and approval before patient enrollment. The trial was conducted in accordance with the Declaration of Helsinki.

A single oral dose of cefpodoxime suspension (4 mg/kg) was administered 2, 3, 4 or 6 h before planned MEF collection. The MEF was aspirated, under general anesthetic, into a Juhn Tym-tap® fluid collection tube. When clinically indicated both left and right ears were sampled individually. The nature of the exudate was recorded and samples were assayed separately. Within 10 min of MEF sampling, a blood sample was obtained (by finger stick) to determine corresponding serum concentrations of cefpodoxime. MEF and serum samples were stored at −20°C before assay.

All samples were assayed microbiologically as follows. Quantitation was based on the diffusion of samples from wells into nutrient agar medium. The agar medium was seeded with either *Sarcina lutea* (standard range, 0.5 to 16 mg/l) or *Providencia rettgeri* (0.015 to 0.5 mg/l). Before placement into the agar wells the MEF samples were weighed. The weight of the sample was determined by weighing the Juhn Tym-tap® tube including MEF. The MEF was then removed, the tube

oroughly rinsed, dried and reweighed. The difference in the eights was attributed to the MEF sample. The sample was ser ted with phosphate buffer (pH 6.0), vortexed and (hout further manipulation. The concentration of e me was determined by a calculation using the equation: Concentration$_{assay}$ × (volume$_{dilution}$ + weight$_{sample}$) + eight$_{sample}$.

Results. Twenty-four children whose ages ranged from 5 months to 9 years (34.13 ± 26.69 months) and weighing 7.1 to 2 kg (13.29 ± 4.49 kg) received cefpodoxime. MEF samples ere obtained from 22 patients and serum samples obtained rom all 24 children. Thirty-three MEF samples were ana-yzed. The cefpodoxime concentrations in MEF and serum are iven in Table 1. The appearance of the MEF, whether serous, urulent or mucopurulent, was noted at the time of MEF mpling. There was no correlation between the appearance the fluid and the MEF concentration of cefpodoxime.

Some variability in the penetration of cefpodoxime into the EF was observed. There were three children (12%) in whom e concentrations of cefpodoxime in the MEF, when mea-ed at various times, were <0.01 μg/ml. Because the MIC$_{90}$ ainst penicillin-susceptible strains is 0.03 to 0.06 μg/ml, it possible that these children would respond to therapy.

The wide confidence intervals reflect the design of the udy in that ethically it is practically impossible to collect ltiple specimens from individual children which would ve reduced the confidence intervals.

Discussion. Cefpodoxime proxetil is deesterified in the testine to the active metabolite cefpodoxime which is the e acid form of the prodrug. Approximately 50% of this ive metabolite is available to exert an antimicrobial effect th the remaining unabsorbed cefpodoxime proxetil being in the feces and urine.[6,7] The peak plasma concen-(C_{max}) after administration of a single oral dose of 200 g of cefpodoxime to healthy young volunteers in six different udies ranged from 2.1 to 3.1 mg/l occurring 2 to 3 h postdose. ne area under the concentration/time curve ranged from 1.8 to 14.5 mg/l/h. The elimination half-life varied from 2.2 to 9 h.[4]

For an antimicrobial drug to be effective in the treatment AOM it is important that the drug not only reach adequate ncentrations in the MEF but also have good activity against e causative pathogens of AOM.

There are a number of antibiotics that penetrate the MEF. iddle ear fluid values for cefixime 2 to 3 h after the dministration of a single oral dose of 8 mg/kg, although arying considerably, averaged 14% of serum values, which nged from 0.76 to 33 μg/ml.[8] Loracarbef MEF concentrations 2 h after administration of a single oral dose of 7.5 g/kg were 2 ± 2.6 mg/l equivalent to 48% of plasma ncentrations and 3.9 ± 2.6 mg/l (42% of plasma concentrations) after oral administration of 15 mg/kg.[9] A study by limek et al.[10] showed the mean serum and MEF values of lfamethoxazole 20 mg/kg 1 to 3 h after administration of a ngle oral dose to be 44.6 and 8.2 μg/ml, respectively, hereas the levels obtained when a single dose of sulfamethoxazole was administered in combination with 4 mg/kg rimethoprim were 2.03 and 1.39 μg/ml, respectively. Ampillin concentrations in MEF and serum sampled 27 min to h postadministration of 30 mg/kg as a single dose were 0.18 0.52 and 3.5 to 9.5 μg/ml, respectively.[11]

From the results obtained in this study it is evident that ime penetrates MEF reaching a peak concentration 0.67 μg/ml 2 h after a single oral dose of 4 mg/kg. centrations of cefpodoxime reached in the MEF at 6 h ed the MIC$_{90}$ of penicillin-susceptible *S. pneumoniae* ≤0.06 μg/ml) and *H. influenzae* (MIC$_{90}$: ≤0.06 μg/ml, beta-lactamase-negative; and ≤0.06 μg/ml, beta-lactamase positive).[12] In addition the MIC$_{90}$ for intermediately penicillin-resistant pneumococci (0.25 μg/ml) and for *M. catarrhalis* (0.25 μg/ml) were also exceeded at 6 h.[13] However, cefpodoxime did not reach the MIC required for the treatment of AOM caused by penicillin-resistant pneumococci (MIC ≥ 2 μg/ml).

The maximum serum concentration of 2.64 ± 0.51 μg/ml observed at a t_{max} of 2 h in this study is, as expected after a 4-mg/kg dose, slightly higher than that obtained by Fujii et al.[14] (C_{max} 2.24 μg/ml) in pediatric patients who received 3 mg/kg cefpodoxime under fasting conditions.

TABLE 1. Concentrations of cefpodoxime in middle ear fluid and serum

Time	Patient	Serum Concentration (μg/ml)	MEF Concentration (μg/ml)
2 h	1	3.00	*
	7	3.00	1.11
	9	1.70	1.28
			1.90
	10	2.80	0.03
			<0.01
	18	3.00	0.91
n	5	5	6
Mean		2.64	0.87
SD		0.51	0.67
Range		1.70–3.00	<0.01–1.90
Mean of ratio (mean ± SD)			0.43 ± 0.40
3 h	2	2.80	1.00
			1.76
	4	2.25	1.57
			0.10
	5	1.80	<0.01
			<0.01
	6	1.60	0.89
			0.22
	20	1.90	1.39
	22	3.20	0.85
	23	3.80	1.19
n	7	7	11
Mean		2.48	0.82
SD		0.75	0.61
Range		1.60–3.80	<0.01–1.76
Mean of ratio (mean ± SD)			0.34 ± 0.26
4 h	11	2.25	<0.01
			0.14
	12	1.40	0.75
			0.57
	13	2.70	0.61
			0.63
	16	*	*
	19	2.00	0.73
	21	1.70	0.49
n	6	5	8
Mean		2.01	0.49
SD		0.45	0.25
Range		1.40–2.70	<0.01–0.75
Mean of ratio (mean ± SD)			0.26 ± 0.16
6 h	3	1.35	0.16
			0.31
	8	1.20	0.45
			0.55
	14	1.70	0.87
	15	0.47	0.33
	17	0.95	0.76
	24	0.40	0.79
n	6	6	8
Mean		1.01	0.52
SD		0.46	0.24
Range		0.40–1.70	0.16–0.87
Mean of ratio (mean ± SD)			0.64 ± 0.54

* No sample.

The MEF:serum ratio varied over time between 0.26 and 0.64. The highest MEF:serum ratio found at 6 h suggests that the drug persists in the MEF longer than in serum. This is an important finding because the activity of beta-lactams is related to the time above MIC and these data suggest that the drug concentration remains above the MIC for susceptible pathogens for at least 6 h.

The bioavailability of cefpodoxime is increased when the antibiotic is administered together with food, with the concentration/time curve and C_{max} increasing by up to 34 and 22%, respectively.[15] Because all patients received cefpodoxime under fasting conditions, it may be that the concentration of cefpodoxime reached in MEF would be increased when the drug is administered to the patient with food.

Our data show that cefpodoxime at 4 mg/kg penetrates middle ear fluid, reaching concentrations exceeding the MIC_{90} for many common pathogens, but not for penicillin-resistant pneumococci, causing acute otitis media in children.

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1. Klein JO. Selection of oral antimicrobial agents for otitis media and pharyngitis. Infect Dis Clin Prac 1994;3:151–7.
2. Baquero F, Loza E. Antibiotic resistance of microorganisms involved in ear, nose and throat infections. Pediatr Infect Dis J 1994;13:S9–14.
3. Nelson TC, Mason EO, Kaplan SL. Activity of oral antibiotics in middle ear and sinus infections caused by penicillin-resistant *Streptococcus pneumoniae*: implications for treatment. Pediatr Infect Dis J 1994;13:585–9.
4. Frampton JE, Brogden RN, Langtry HD, Buckley MM. Cefpodoxime proxetil: a review of its antibacterial activity, pharmacokinetic properties and therapeutic potential. Drugs 1992;44:889–917.
5. Klein JO. Otitis media. Clin Infect Dis 1994;19:823–33.
6. Tremblay D, Dupront A, Ho C, Coussediere D, Lenfant B. Pharmacokinetics of cefpodoxime in young and elderly volunteers after single doses. J Antimicrob Chemother 1990; 26(Suppl. E):21–8.
7. Borin MT. A review of the pharmacokinetics of cefpodoxime proxetil. Drugs 1991;42(Suppl. 3):13–21.
8. Wiedermann BL, Schwartz RH. Effect of blood contamination on the interpretation of antibiotic concentrations in the middle ear. Pediatr Infect Dis J 1992;11:244–5.
9. Kusmiesz H, Shelton S, Brown O, Manning S, Nelson JD. Loracarbef concentrations in the middle ear fluid. Antimicrob Agents Chemother 1990;34:2030–1.
10. Klimek JJ, Bates TR, Nightingale C, et al. Penetration characteristics of trimethoprim-sulfamethoxazole in middle ear fluid of patients with chronic serous otitis media. J Pediatr 1980;96:1087–9.
11. Voelker MS, Nightingale CH, Quintiliani R, et al. Sultamicillin use in otitis media: penetration of sulbactam, a beta-lactamase inhibitor, and ampicillin into middle ear fluid. Curr Ther Res 1985;38:738–45.
12. Jones RN, Barry AL. Antimicrobial activity and disk diffusion susceptibility testing of U-76 253A (R-3746), the active metabolite of the new cephalosporin ester, U-76 252 (CS-807). Antimicrob Agents Chemother 1988;32:443–9.
13. Spangler SK, Jacobs MR, Panku GA, Appelbaum PC. Susceptibility of 170 penicillin-susceptible and penicillin-resistant pneumococci to 6 oral cephalosporins, 4 quinolones, desacetyl cefotaxime, Ro 23-9424 and RP 64829. J Antimicrob Chemother 1993;31:273–80.
14. Fujii R, Meguro H, Arimasu O, Shiraishi H, Abe T. Overall clinical evaluation of cefpodoxime proxetil against infections in paediatric fields. Jpn J Antibiot 1989;42:1439–53.
15. Hughes GS, Heald DL, Barber BK, et al. The effects of gastric pH on the pharmacokinetics of a new oral cephalosporin, cefpodoxime proxetil. Clin Pharm Ther 1989;12:674–85.

CONGENITAL BRUCELLOSIS IN A PREMATURE INFANT

Brucellosis is a febrile illness caused by brucellae. Nucleic acid hybridization studies indicate that only one species of *Brucella* exists; however, the genus has historically been divided into species based on the natural animal hosts.[1] Brucellosis is primarily a disease of animals. Human infection is usually acquired by contact with infected animals.[2, 3] The acquisition of brucellosis by food contamination has been growing in incidence.[4] The major reservoirs include goats and sheep (*Brucella melitensis*), swine (*Brucella suis*) and cattle (*Brucella abortus*). These Gram-negative coccobacilli remain viable in fomites or water for many days before transmission by ingestion of infected milk, cheese products or meat.[4, 5] Although brucellosis is an important human infection in many parts of the world, human to human transmission, especially perinatal transmission, is uncommon.[2, 6] We report one case of perinatal transmission of *B. melitensis* from an infected mother to her preterm infant.

Case report. A 17-year-old primigravida with a history of one prenatal visit, presented to our hospital with malaise, abdominal cramps and vaginal bleeding. "Flu"-like symptoms had recently occurred in her family members. She was febrile (38.6°C) and had ruptured fetal membranes. A complete blood count (CBC) revealed an elevated fraction of segmented and immature neutrophils. Blood cultures were drawn and she was treated with intravenous ampicillin and gentamicin. Three days later she went into preterm labor and delivered vaginally a viable 24-week 650-g female infant. Apgar scores were 7 at 1 and 5 min.

Histopathologic examination of the placenta by a pediatric pathologist showed acute and chronic chorioamnionitis and funisitis. A placental Gram-stained smear was negative, but a culture was positive for coagulase-negative *Staphylococcus*.

Because of the infant's extreme prematurity, she was intubated soon after birth, given prophylactic surfactant and placed on synchronous intermittent mandatory ventilation. Pertinent findings on physical examination were significant hypotension requiring vasopressors, a systolic grade II heart murmur, a 4- by 2-cm midline abdominal mass (later found to be a hematoma) and small infarctions of the distal phalanx of

40% of the dosing interval with *H. influenzae* and penicillin-susceptible *S. pneumoniae* are shown in Table 1. Pharmacokinetic data and the MIC_{90}s were obtained from results published in the literature [Doern et al, 1988; Spangler et al, 1994; Craig, 1996; Meyers et al, 1996]. The resulting time above MIC values are slightly different from those previously published for pediatric dosing regimens [Craig and Andes, 1996]. Against *S. pneumoniae*, concentrations of amoxicillin are above the MIC_{90} for 100% of the time. All the oral cephalosporins provide levels above the MIC of penicillin-susceptible strains for at least 50% of the dosing interval. Thus, one would expect that all these oral agents would produce similar efficacy against such strains. In contrast, several of the oral cephalosporins provide levels above the MIC_{90} for less than 40% of the dosing interval with *H. influenzae*. The value for amoxicillin-clavulanate is 45%, whereas the two cephalosporins with the lowest MIC values, cefixime and cefpodoxime, are above the MIC_{90} for the entire dosing interval.

Table 2 shows the duration of time that serum levels exceed the MIC_{50} and MIC_{90} of penicillin-intermediate and -resistant strains of *S. pneumoniae*. Amoxicillin appears to be the most potent agent against intermediate strains, with cefprozil, cefuroxime, and cefpodoxime also demonstrating reasonable times above MIC. Against resistant strains, however, only amoxicillin is likely to prove effective based on time above MIC data. The use of oral cephalosporins with shorter times above the MIC may contribute to the continuing emergence of resistant strains.

The time above MIC is also the important pharmacokinetic/pharmacodynamic parameter for the macrolide antimicrobials. As shown in Table 3, the bactericidal efficacy of erythromycin and clarithromycin against susceptible strains of *S. pneumoniae* in patients with acute otitis media was 93 and 100%, respectively [Howie, 1992; Klein, 1993]. The standard doses used in these studies produce serum levels that exceed the MIC_{90} for 88 to 100% of the dosing interval. In contrast, the bactericidal efficacy of these drugs in patients infected with *H. influenzae* was only 15 to 20%. These poor results are not unanticipated considering that serum concentrations never exceed the high MIC_{90} values for *H. influenzae*. Although it is often argued that it is the tissue levels, rather than the serum concentrations, of the macrolides that must be taken into account, it is also important to remember that the majority of the drug is located intracellularly in the tissues whereas the organism is primarily located extracellularly.

NEW DOSAGE REGIMENS

The aims of new dosage regimens are to maximize both the time above MIC and compliance. Although increasing the dosing frequency may enhance the duration of time serum levels exceed the MIC, frequent dosing can also reduce compliance and thus defeat the original therapeutic goals. The simplest new dosing regimen is to use higher doses with the same dosing frequency. This method is currently being employed in France and other parts of Europe. Another possibility is to use higher doses but with a reduced dosing frequency. For example, instead of using amoxicillin in children at 40 mg/kg/day divided in three doses (approximately 13 mg/kg every 8 hours), the daily dose is doubled to 80 mg/kg/day but only given every 12 hours (each dose 40 mg/kg). This results in a tripling of the single dose and a prolonged time above the MIC. In this way, compliance and the time above MIC can both be improved. Unfortunately, however, these changes in dosing are not possible with all drugs and some require an increase in the dose combined with an increase in the dosing frequency.

TABLE 1 Time Above MIC (Time > MIC) for Oral β-Lactam Antibiotics Against Penicillin-Susceptible *S. pneumoniae* and *H. influenzae*[a]

		S. pneumoniae		*H. influenzae*	
Drug	Regimen	MIC_{90}	Time > MIC	MIC_{90}	Time > MIC
Amoxicillin/ clavulanate	500 mg q8h	0.06	100%	2	43%
Cefaclor	500 mg q8h	0.5	60%	8	20%
Cefuroxime	500 mg q12h	0.12	75%	2	35%
Cefprozil	500 mg q12h	0.25	75%	8	21%
Loracarbef	400 mg q12h	0.5	50%	8	17%
Cefpodoxime	200 mg q12h	0.25	83%	0.12	100%
Cefixime	400 mg q24h	0.5	59%	0.06	100%

[a]Percentages based on calculations with data from [Craig, 1996; Doern et al, 1988; Meyers et al, 1996; and Spangler et al, 1994].

TABLE 2 Time Above MIC for Oral β-Lactam Antibiotics Against Penicillin-Intermediate (I) and -Resistant (R) *S. pneumoniae*[a]

		S. pneumoniae (I)		*S. pneumoniae* (R)	
Drug	Regimen	MIC_{50-90}	Time > MIC	MIC_{50-90}	Time > MIC
Amoxicillin	500 mg every 8 hours	0.25–1	80–55%	1–2	55–43%
Cefaclor	500 mg every 8 hours	8–16	20–0%	32–64	0%
Cefuroxime	500 mg every 12 hours	0.5–2	55–35%	4–8	25–0%
Cefprozil	500 mg every 12 hours	0.5–4	64–32%	4–16	32–0%
Loracarbef	400 mg every 12 hours	2–16	33–0%	16	0%
Cefpodoxime	200 mg every 12 hours	0.25–2	83–21%	2–4	21–0%
Cefixime	400 mg every 24 hours	4–16	0%	32–64	0%

[a]Percentages based on calculations with data from Craig, 1996; Doern et al, 1988; Meyers et al, 1996; and Spangler et al, 1994.

NEW ANTIMICROBIALS

The majority of the fluoroquinolones currently on the market have a ratio between the 24-hour AUC and the MIC that is insufficient to ensure maximum efficacy against *S. pneumoniae* [Craig and Andes, 1995]. However, some of the newer agents in this ... which are currently undergoing clinical trials, ...ir to have lower MIC values (MIC_{90} = 0.06–0.5 ...mL) and so should have more effective AUC: MIC ratios. Other new orally administered antimicrobials with low MIC values include the naphthyridones (MIC_{90} = 0.12–0.25 μg/mL), the glycylcyclines (MIC_{90} = 0.12–0.5 μg/mL), the oxazolidinones (MIC_{90} = 0.5–1.0 μg/mL), the ketolides (MIC_{90} = 0.016–0.25 μg/mL), and the streptogramins (MIC_{90} = 0.25–2.0 μg/mL).

THE FUTURE

A number of areas require further research to control antimicrobial resistance. For example, although it is recognized that quinolones with a high peak:MIC ratio (greater than 8) can reduce the emergence of resistant organisms, very little data are available on the pharmacokinetic/pharmacodynamic parameters for other antimicrobials that might contribute to such a reduction. The impact of exposure to alternating high and low drug concentrations on the emergence of lysis-deficient mutant strains of pneumococci is another area of research that should expand our knowledge on antimicrobial resistance. In addition, further data are required on the short- and long-term effects of therapy with different drugs on the nasopharyngeal flora and colonization with various resistant organisms. There might be differences in these effects between bactericidal drugs and those that produce only a bacteriostatic effect.

The prudent use of antimicrobial agents is a necessary control measure to reduce resistance. Shorter courses of therapy and less prophylactic use may be ways to reduce antimicrobial use for respiratory infections. Optimal infection control practices in day care settings and hospitals can reduce the spread of resistant organisms. Lastly, there is increasing interest in agents such as vaccines that could reduce attachment of bacteria to the mucosa of the nasopharynx and thereby reduce colonization and subsequent disease.

TABLE 3 Relationship Between Time Above MIC and Bacteriologic Cure of Macrolide Antibiotics in Acute Otitis Media[a]

	S. pneumoniae		*H. influenzae*	
...arameters	Erythromycin	Clarithromycin	Erythromycin	Clarithromycin
...90	0.06	0.06	8	4
Time > MIC	88%	100%	0%	0%
Bacteriologic cure	14/15 (93%)	12/12 (100%)	3/20 (15%)	3/15 (20%)

[a]Data obtained from Craig and Andes, 1996; Howie, 1992; and Klein, 1993.

REFERENCES

Andes D, Urban A, Craig WA (1995) In-vivo activity of amoxicillin and amoxicillin-clavulanate against penicillin-resistant pneumococci. Abstract A82. In *Proceedings and Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC: American Society for Microbiology.

Craig WA, Ebert SC (1991) Killing and growth of bacteria in vitro: A review. *Scand J Infect Dis* 74:S63–70.

Craig W, Rikardsdottir S, Watanabe Y (1992) In-vivo and in-vitro postantibiotic effects (PAEs) of azithromycin, Abstract. In *Program and Abstracts of the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC: American Society for Microbiology.

Craig WA, Ebert S, Watanabe Y (1993) Differences in time above MIC required for efficacy of beta-lactams in animal infection models. Abstract 86. In *Proceedings and Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC: American Society for Microbiology.

Craig WA (1995) Interrelationship between pharmacokinetics and pharmacodynamics in determining dosage regimens for broad-spectrum cephalosporins. *Diagn Microbiol Infect Dis* 22:89–96.

Craig WA, Andes DR (1995) Parenteral versus oral antibiotic therapy. *Med Clin N Amer* 79:497–508.

Craig WA, Andes D (1996) Pharmacokinetics and pharmacodynamics of antibiotics in otitis media. *Pediatr Infect Dis J* 15:255–259.

Craig WA (1996) Penicillins. In *Current Infectious Disease Drugs*, 1st edition. Ed., V. Andriole. Philadelphia: Current Medicine, pp 62–104.

Dagan R, Abramson O, Leibovitz E, Lang R, Goshen S, Greenberg D, Yagupsky P, Leiberman A, Fliss DM (1995) Impaired bacteriologic response to oral cephalosporins in acute otitis media (AOM) caused by intermediately penicillin-resistant pneumococci (I-PRP). Abstract LB-25. In *Proceedings and Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC: American Society for Microbiology.

Doern GV, Jorgensen JH, Thornsberry C (1988) National collaborative study of the prevalence of antimicrobial resistance among clinical isolates of *Haemophilus influenzae*. *Antimicrob Agents Chemother* 32:180–185.

Ebert SC, Craig WA (1990) Pharmacodynamic properties of antibiotics: Application to drug monitoring and dosage regimen design. *Infect Control Hosp Epidemiol* 6:319–326.

Howie VM, Dillard KRD, Lawrence B (1985) *In vivo* sensitivity test in otitis media: Efficacy of antibiotics. *Pediatr* 75:8–13.

Howie V (1992) Eradication of bacterial pathogens from middle ear infections. *Clin Infect Dis* 14:S209–S211.

Klein JO (1993) Microbiologic efficacy of antibacterial drugs for acute otitis media. *Pediatr Infect Dis J* 12:973–975.

Leggett JE, Fantin B, Ebert S, Totsuka K, Vogelman B, Calame W, Mattie H, Craig WA (1989) Comparative antibiotic dose-effect relations at several dosing intervals in murine pneumonitis and thigh-infection models. *J Infect Dis* 135:217–223.

Leggett JE, Ebert S, Fantin B, Craig WA (1991) Comparative dose-effect relations at several dosing intervals for beta-lactams, aminoglycoside and quinolone antibiotics against gram-negative bacilli in murine thigh-infection and pneumonitis models. *Scand J Infect Dis* 74:S179–S184.

Meyers BR, Gurtman AC, Farrington JM (1996) Cephalosporins. In *Current Infectious Disease Drugs*, 1st edition. Ed., V. Andriole. Philadelphia: Current Medicine, pp 13–61.

Moine P, Vallee E, Azoulay-Dupouis E, Bourget P, Bedos J-P, Bauchet J, Pocidalo J-J (1994) In vivo efficacy of a broad-spectrum cephalosporin, ceftriaxone, against penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae* in a mouse pneumonia model. *Antimicrob Agents Chemother* 38:1953–1958.

Spangler SK, Jacobs MR, Appelbaum PC (1994) *In vitro* susceptibilities of 185 penicillin-susceptible and -resistant pneumococci to WY 49605 (SUN/SY 5555), a new oral penem, compared with those to pencillin G, amoxicillin, amoxicillin-clavulanate, cefixime, cefaclor, cefpodoxime, cefuroxime, and cefdinir. *Antimicrob Agents Chemother* 38:2902–2904.

Urban A, Andes D, Craig WA (1995) *In-vivo* activity of cefpodoxime against penicillin-resistant pneumococci. Abstract 2229. In *Final Program and Abstracts of the 19th International Congress of Chemotherapy*. Ottawa, Canada: Canadian Infectious Disease Society.

Vogelman B, Gudmundsson S, Leggett J, Turnidge J, Ebert S, Craig WA (1988) Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. *J Infect Dis* 158:831–847.