

Antimicrobial Resistance among Clinical Isolates of *Streptococcus pneumoniae* in the United States during 1999–2000, Including a Comparison of Resistance Rates since 1994–1995

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A total of 1,531 recent clinical isolates of *Streptococcus pneumoniae* were collected from 33 medical centers nationwide during the winter of 1999–2000 and characterized at a central laboratory. Of these isolates, 34.2% were penicillin nonsusceptible (MIC \geq 0.12 μ g/ml) and 21.5% were high-level resistant (MIC \geq 2 μ g/ml). MICs to all beta-lactam antimicrobials increased as penicillin MICs increased. Resistance rates among non-beta-lactam agents were the following: macrolides, 25.2 to 25.7%; clindamycin, 8.9%; tetracycline, 16.3%; chloramphenicol, 8.3%; and trimethoprim-sulfamethoxazole (TMP-SMX), 30.3%. Resistance to non-beta-lactam agents was higher among penicillin-resistant strains than penicillin-susceptible strains; 22.4% of *S. pneumoniae* were multiresistant. Resistance to vancomycin and quinupristin-dalfopristin was not detected. Resistance to rifampin was 0.1%. Testing of seven fluoroquinolones resulted in the following rank order of in vitro activity: gemifloxacin > sitafloxacin > moxifloxacin > gatifloxacin > levofloxacin = ciprofloxacin > ofloxacin. For 1.4% of strains, ciprofloxacin MICs were \geq 4 μ g/ml. The MIC_{90s} (MICs at which 90% of isolates were inhibited) of two ketolides were 0.06 μ g/ml (ABT773) and 0.12 μ g/ml (telithromycin). The MIC₉₀ of linezolid was 2 μ g/ml. Overall, antimicrobial resistance was highest among middle ear fluid and sinus isolates of *S. pneumoniae*; lowest resistance rates were noted with isolates from cerebrospinal fluid and blood. Resistant isolates were most often recovered from children 0 to 5 years of age and from patients in the southeastern United States. This study represents a continuation of two previous national studies, one in 1994–1995 and the other in 1997–1998. Resistance rates with *S. pneumoniae* have increased markedly in the United States during the past 5 years. Increases in resistance from 1994–1995 to 1999–2000 for selected antimicrobial agents were as follows: penicillin, 10.6%; erythromycin, 16.1%; tetracycline, 9.0%; TMP-SMX, 9.1%; and chloramphenicol, 4.0%, the increase in multiresistance was 13.3%. Despite awareness and prevention efforts, antimicrobial resistance with *S. pneumoniae* continues to increase in the United States.

Antimicrobial resistance with *Streptococcus pneumoniae* was first recognized in 1917, when optochin (ethylhydrocupreine) was used to treat infections caused by *S. pneumoniae*, and resistance developed while patients were on therapy (13). Fifty years later, the first isolate of penicillin-nonsusceptible *S. pneumoniae* of recognized clinical significance (MIC = 0.5 μ g/ml) was recovered in Australia (9). In the United States, the first report of infection due to a non-penicillin-susceptible isolate of *S. pneumoniae* (MIC = 0.25 μ g/ml) was in 1974. The patient had pneumococcal meningitis and did not respond to penicillin therapy, even when high doses of this agent were administered (15).

Antimicrobial resistant *S. pneumoniae* became widespread in many parts of the world during the 1980s (13). In the United States, however, resistance first became manifest during the early part of the decade of the 1990s. During the 1980s two national surveillance studies revealed overall penicillin resistance rates in the United States to be at levels of 3 to 5%, and

importantly, where resistance was observed, it was typically only of the intermediate level (11, 24). By 1991–1992, however, overall penicillin resistance rates (intermediate plus resistant [I + R]) had jumped to 17.8% in the United States (27).

A national surveillance study conducted in 1994–1995 with 30 United States medical centers reported *S. pneumoniae* overall penicillin resistance at 23.6%; multiresistance was 9.1% (5). In 1997–1998, this surveillance project was repeated, with 24 of the 1994–1995 medical centers participating plus an additional 10 centers. Results of the 1997–1998 study indicated that overall rates of penicillin resistance had increased to 29.5% and the rate of multiresistance was 16.0% (6). The present report describes the results of a third surveillance project, performed in 1999–2000. This study included 33 of the 34 centers participating in the 1997–1998 study. Twenty-two medical centers have been participants in all three national surveillance studies, which provides an opportunity for evaluation of resistance rates among a common group of medical centers over a 5-year period of time in the United States.

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MATERIALS AND METHODS

Unique patient isolates of *S. pneumoniae* were collected in each of 33 United States medical center microbiology laboratories from November 1, 1999, through

TABLE 1. In vitro activities of 32 antimicrobial agents for 1,531 isolates of *S. pneumoniae*

Antimicrobial	All strains (n = 1,531)										Penicillin-susceptible strains (n = 1,008)										Penicillin-intermediate strains (n = 194)										Penicillin-resistant strains (n = 329)									
	MIC ₅₀		MIC range		% I	% R	% I + R	MIC ₅₀		MIC range		% I	% R	% I + R	MIC ₅₀		MIC range		% I	% R	% I + R	MIC ₅₀		MIC range		% I	% R	% I + R												
Penicillin	0.03	2	≤0.015-32	12.7	21.5	34.2	0.015	0.03	0.12	0.03	0.12	0.01	0.1	0.1	0.1	0.1	0.12-32	13.9	32.0	45.9	8	16	1-32	0.6	99.1	99.7														
Amoxicillin ^a	0.015	2	≤0.004-8	4.2	2.2	6.3	0.015	0.03	0.06	0.06	0.06	0.0	0.0	0.0	0.25	1	0.03-2	0.0	0.0	0.0	2	4	0.5-8	19.5	10.0	29.5														
Amoxicillin-clavulanate ^b	0.015	2	≤0.004-8	4.6	1.7	6.3	0.015	0.03	0.06	0.06	0.06	0.0	0.0	0.0	0.25	1	0.015-2	0.0	0.0	0.0	2	4	0.5-8	21.3	7.9	29.2														
Cefuroxime ^c	0.03	8	≤0.015-32	2.0	25.3	27.3	0.03	0.12	0.25	0.25	0.25	0.1	0.1	0.1	1	4	0.12-32	13.9	32.0	45.9	8	16	1-32	0.6	99.1	99.7														
Ceftriaxone	0.03	2	≤0.008-8	10.3	14.4	24.7	0.03	0.06	0.06	0.06	0.06	0.0	0.0	0.0	0.25	1	0.03-4	23.7	2.6	26.3	2	4	0.5-8	33.7	65.7	99.4														
Cefepodoxime	0.03	4	≤0.015-32	2.0	25.7	27.7	0.03	0.06	0.06	0.06	0.06	0.5	0.1	0.6	0.5	2	0.03-16	11.9	34.0	45.9	4	8	1-32	0.6	99.4	100.0														
Cefixime	0.25	32	≤0.06->128				0.25	0.5	0.5	0.5	0.5	0.06-16			4	16	0.25-64				32	64	4->128																	
Cefprozil	0.25	16	≤0.03-64	1.3	24.8	26.1	0.12	0.25	0.25	0.25	0.25	0.03-16	0.0	0.1	0.1	2	8	0.12-16	9.3	26.3	35.6	16	32	4-64	0.6	99.4	100.0													
Cefaclor	0.5	64	≤0.06->128	2.9	29.5	32.4	0.5	1	1	1	1	1.2	0.2	1.4	4	64	0.25-64	16.5	62.4	78.9	64	>128	8->128	0.0	100.0	100.0														
Loracarbef	1	128	≤0.06->128	2.7	29.3	32.0	1	2	2	2	2	0.06-128	0.8	0.1	0.9	8	64	0.25-128	17.5	60.8	78.4	128	>128	16->128	0.0	100.0	100.0													
Cefditoren	0.015	0.5	≤0.008-4				0.015	0.03	0.03	0.03	0.03	0.008-0.5			0.12	0.5	≤0.008-2				0.5	2	0.12-4																	
Cefdinir	0.06	8	≤0.008->8	1.4	25.8	27.2	0.06	0.12	0.12	0.12	0.12	0.008-0.5	0.0	0.0	0.0	0.5	4	0.03-8	10.8	34.5	45.4	4	6	0.5->8	0.0	99.7	99.7													
Clarithromycin	0.06	8	≤0.03->64	0.9	25.2	26.1	≤0.03	0.06	0.06	0.06	0.06	0.03->64	0.6	5.3	5.9	0.06	>64	≤0.03->64	1.0	41.8	42.8	4	>64	≤0.03->64	1.8	76.3	78.1													
Erythromycin	0.06	8	≤0.03->64	0.5	25.7	26.2	0.06	0.12	0.12	0.12	0.12	0.03->64	0.5	5.6	6.1	0.06	>64	≤0.03->64	0.5	42.3	42.8	4	>64	≤0.03->64	0.3	77.8	78.1													
Azithromycin	0.12	16	≤0.03->64	0.5	25.7	26.2	0.12	0.25	0.25	0.25	0.25	0.03->64	0.4	5.7	6.1	0.25	>64	≤0.03->64	0.0	42.8	42.8	8	>64	0.06->64	0.9	77.2	78.1													
Clindamycin	0.06	0.12	≤0.008->8	0.3	8.9	9.2	0.06	0.12	0.12	0.12	0.12	0.008->8	0.2	1.3	1.5	0.12	>8	0.03->8	0.5	19.6	20.1	0.12	>8	0.03->8	0.3	25.8	26.1													
Tetracycline	0.12	16	≤0.03-64	0.3	16.3	16.6	0.12	0.12	0.12	0.12	0.12	0.03-32	0.2	3.1	3.3	0.12	32	0.06-64	0.5	31.4	31.9	0.25	32	0.06-64	0.3	48.0	48.3													
Chloramphenicol	4	4	≤0.5->16				8.3	8.3	2	4	4	0.5->16	1.0	1.0	1.0	4	8	≤0.5->16				4	16	1->16																
TMP-SMX	0.25	8	≤0.03-32	5.6	30.3	35.9	0.25	1	1	1	1	0.03-32	5.6	7.6	13.2	0.5	8	≤0.03-16	9.3	39.2	48.5	8	16	0.25-32	3.3	94.5	97.9													
Rifampin	≤0.12	≤0.12	≤0.12->4	0.0	0.1	0.1	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	0.12-0.25	0.0	0.0	0.0	0.0	≤0.12	≤0.12-0.25	0.0	0.0	0.0	≤0.12	≤0.12	≤0.12->4	0.0	0.3	0.3													
Vancomycin	0.5	0.5	≤0.015-0.5	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.015-0.5	0.0	0.0	0.0	0.5	0.5	0.06-0.5	0.0	0.0	0.0	0.5	0.5	0.25-0.5	0.0	0.0	0.0													
Quinupristin-dalfopristin	0.25	0.5	≤0.008-2	0.1	0.0	0.1	0.25	0.5	0.5	0.5	0.5	0.008-2	0.1	0.0	0.1	0.25	0.5	0.06-1	0.0	0.0	0.0	0.25	0.5	0.12-1	0.0	0.0	0.0													
Ofloxacin	2	2	0.12->64	5.0	0.6	5.6	2	2	2	2	2	0.12-32	5.8	0.3	6.1	2	2	0.25->64	4.6	1.0	5.7	2	2	1-16	2.7	1.2	4.0													
Ciprofloxacin	1	1	0.06->64				1	2	2	2	2	0.06-32				1	1	0.12->64				1	1	0.25-16																
Levofloxacin	1	1	0.12-64	0.4	0.3	0.7	1	1	1	1	1	0.12-16	0.3	0.2	0.5	1	1	0.25-64	0.0	1.0	1.0	1	1	0.5-8	0.9	0.3	1.2													
Gatifloxacin ^d	0.25	0.25	0.015-16	0.1	0.3	0.4	0.25	0.25	0.25	0.25	0.25	0.015-4	0.0	0.2	0.2	0.25	0.25	0.06-16	0.0	1.0	1.0	0.25	0.25	0.12-2	0.6	0.0	0.6													
Gemifloxacin	0.015	0.03	≤0.008-2				0.015	0.03	0.03	0.03	0.03	0.008-0.25				0.015	0.03	≤0.008-2				0.015	0.03	≤0.008-0.25																
Sifalofacin	0.03	0.06	≤0.008-2				0.03	0.06	0.06	0.06	0.06	0.008-0.25				0.03	0.06	≤0.008-2				0.03	0.06	0.015-0.25																
Moxifloxacin ^d	0.12	0.12	≤0.008-8	0.1	0.1	0.3	0.12	0.12	0.12	0.12	0.12	0.008-2	0.2	0.0	0.2	0.12	0.12	0.03-8	0.0	1.0	1.0	0.12	0.12	0.06-1	0.0	0.0	0.0													
ABT773	≤0.008	0.06	≤0.008-4				≤0.008	0.015	0.015	0.015	0.015	0.008-0.5				≤0.008	0.06	≤0.008-1				0.03	0.25	≤0.008-4																
Telithromycin	0.015	0.12	≤0.004-2				0.015	0.015	0.015	0.015	0.015	0.004-1				0.015	0.12	≤0.004-0.5				0.06	0.5	0.008-2																
Linezolid	1	2	≤0.004-2				1	2	2	2	2	0.004-2				1	2	0.25-2				1	2	0.25-2																

^a Percents I and R based on NCCLS 2000 breakpoints of 4 and ≥8 µg/ml, respectively. Percents I and R based on 1999 breakpoints of 1 and ≥2 µg/ml are 10.6 and 13.6%, respectively.

^b Percents I and R based on NCCLS 2000 breakpoints of 4 and ≥8 µg/ml, respectively. Percents I and R based on 1999 breakpoints of 1 and ≥2 µg/ml are 10.8 and 13.4%, respectively.

^c Percents I and R based on NCCLS 2000 breakpoints of 2 and ≥4 µg/ml, respectively. Percents I and R based on 1999 breakpoints of 1 and ≥2 µg/ml are 1.8 and 27.3%, respectively.

^d Susceptibility breakpoints were adopted by the NCCLS in 2000 and will be published in 2001 documents. Breakpoints are ≤1, 2, ≥4 µg/ml (S, I, R, respectively).

April 30, 2000. Fifty consecutive *S. pneumoniae* isolates were requested from each medical center. At the study centers, pure cultures of *S. pneumoniae* were propagated on 5% sheep blood agar plates, the growth was transferred to a rayon swab, and the swab was placed in a transport tube containing 12 ml of semisolid Ames transport medium with charcoal (Becton Dickinson, Sparks, Md.). Swabs were mailed overnight to the University of Iowa College of Medicine. Only isolates judged by the submitting laboratories as being of clinical significance were included. Demographic data sheets were completed by the contributing medical center and submitted along with every isolate. The following information was obtained: patient medical record number, age, sex, service (inpatient or outpatient), specimen date, and specimen source. Upon receipt at the University of Iowa, isolates were subcultured and isolate identification was verified using conventional criteria. Stock cultures were made using a porous bead system (ProLab Diagnostics, Inc., Austin, Tex.) and stored at -70°C .

Susceptibility testing was performed following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines explicitly (16). Broth microdilution trays were made in-house using Mueller-Hinton broth plus 3% lysed horse blood and were stored at -70°C until use. Thirty-two antimicrobial agents were tested: penicillin, amoxicillin, amoxicillin-clavulanate, cefuroxime, ceftriaxone, cefpodoxime, cefixime, cefprozil, cefaclor, loracarbef, cefditoren, cefdinir, clarithromycin, erythromycin, azithromycin, clindamycin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole (TMP-SMX), rifampin, vancomycin, quinupristin-dalfopristin, ofloxacin, ciprofloxacin, levofloxacin, gatifloxacin, gemifloxacin, sitafloxacin, moxifloxacin, ABT773, telithromycin, and linezolid. Drug powders were obtained from their manufacturers or Sigma-Aldrich (St. Louis, Mo.). *S. pneumoniae* isolates were subcultured twice before susceptibility testing was performed. Broth microdilution trays were inoculated with approximately 5×10^5 CFU of organism/ml (final concentration; 100- μl final volume per well) and incubated for 24 h at 35°C in ambient air before MICs were visually determined.

RESULTS

The in vitro activities of 32 antimicrobial agents against 1,531 *S. pneumoniae* isolates are presented in Table 1. Resistance rates are listed for antimicrobials with NCCLS-approved pneumococcal susceptibility breakpoints. The NCCLS recently changed the breakpoints for *S. pneumoniae* of amoxicillin, amoxicillin-clavulanate, and cefuroxime (17). The breakpoints for amoxicillin and amoxicillin-clavulanate were shifted by two \log_2 dilutions (from ≤ 0.5 , 1, ≥ 2 $\mu\text{g/ml}$ to ≤ 2 , 4, ≥ 8 $\mu\text{g/ml}$ [susceptible {S}, intermediate {I}, resistant {R}, respectively]). Application of the new breakpoints to the current data resulted in a dramatic reduction in the percentage of resistant strains compared to rates determined based on 1999 breakpoints (18), 24.2% versus 6.3%, for both amoxicillin and amoxicillin-clavulanate (Table 1). The breakpoints for cefuroxime were changed by one twofold dilution (from ≤ 0.5 , 1, ≥ 2 $\mu\text{g/ml}$ to ≤ 1 , 2, ≥ 4 $\mu\text{g/ml}$ [S, I, R, respectively]). This resulted in a resistance rate of 29.1% when 2000 breakpoints were applied to the current data, versus 27.3% when old breakpoints were applied. In addition, the NCCLS established susceptibility breakpoints for cefpodoxime, cefprozil, cefaclor, loracarbef, and cefdinir, cephalosporins for which *S. pneumoniae* breakpoints were previously lacking.

Of this collection of *S. pneumoniae* isolates, 34.2% were penicillin nonsusceptible, 12.7% were intermediate (MIC = 0.12 to 1 $\mu\text{g/ml}$), and 21.5% were penicillin resistant (MIC ≥ 2 $\mu\text{g/ml}$) (Table 1). The overall rate of ceftriaxone resistance (I + R) was 24.7%; 14.4% of isolates were resistant. Among the other cephalosporins tested—cefuroxime, cefpodoxime, cefixime, cefprozil, cefaclor, loracarbef, cefditoren and cefdinir—cefditoren was the most active (MIC₉₀ [MIC at which 90% of isolates were inhibited] = 0.5 $\mu\text{g/ml}$) and loracarbef the least active (MIC₉₀ = 128 $\mu\text{g/ml}$). When isolates were grouped

TABLE 2. Multiresistance^a among *S. pneumoniae* isolates, 1999–2000

Penicillin	Resistance				No. (%) of isolates
	Erythromycin	Chloramphenicol	Tetracycline	TMP-SMX	
R	R	S	S	R	125(36.4)
R	R	R	R	R	98(28.6)
R	R	S	R	R	81(23.6)
R	R	S	R	S	15 (4.4)
R	S	R	R	R	13 (3.8)
R	S	S	R	R	5 (1.5)
R	R	R	R	S	5 (1.5)
R	R	R	S	R	1 (0.3)

^a Defined as intermediate or high-level resistance to penicillin plus intermediate or high-level resistance to at least two non-beta-lactam agents. Total number of isolates tested, 1,531; number multiresistant, 343 (22.4%).

according to penicillin susceptibility category, consistently highest rates of resistance to all beta-lactam agents were observed among penicillin-resistant strains. For example, 100% of penicillin-susceptible strains were also susceptible to ceftriaxone, 26.3% of penicillin-intermediate strains were intermediate or resistant to ceftriaxone, and 99.4% of all penicillin-resistant strains were ceftriaxone intermediate or resistant. A similar relationship was seen between penicillin and each beta-lactam tested in this study.

Three macrolide agents were examined: erythromycin, clarithromycin, and azithromycin. While NCCLS breakpoints differ for each macrolide, the resistance rates (I + R) are virtually identical: 0.5 to 0.9% intermediate and 25.2 to 25.7% resistant for all three macrolides (Table 1). In this collection of *S. pneumoniae* isolates, 394 (25.7%) were characterized as erythromycin resistant (MICs ≥ 1 $\mu\text{g/ml}$); erythromycin MICs were 1 to 32 $\mu\text{g/ml}$ for 262 (66.5%) of these 394 isolates and ≥ 64 $\mu\text{g/ml}$ for the remaining 132 strains.

Two principal mechanisms of erythromycin resistance are known to exist among isolates of *S. pneumoniae*: a ribosomal methylase encoded by *ermB*, and an efflux pump encoded by *mefA* (10, 20, 21, 22, 25, 28). Generally, strains with the *ermB* gene have erythromycin MICs of ≥ 64 $\mu\text{g/ml}$ and clindamycin MICs of ≥ 8 $\mu\text{g/ml}$ and are also resistant to streptogramin B agents, a phenotype referred to as MLS_B. In contrast, *mefA*-positive strains have erythromycin MICs of 1 to 32 $\mu\text{g/ml}$ and clindamycin MICs of ≤ 0.25 $\mu\text{g/ml}$. Table 2 describes the patterns of erythromycin and clindamycin susceptibility for the 394 erythromycin-resistant *S. pneumoniae* isolates tested in this study. Of 262 strains for which erythromycin MICs were 1 to 32 $\mu\text{g/ml}$, for 255 (97.3%) clindamycin MICs were ≤ 0.25 $\mu\text{g/ml}$. The phenotype of these isolates was consistent with *mefA*-mediated efflux. For 128 (97.0%) of 132 isolates for which erythromycin MICs were ≥ 64 clindamycin MICs were ≥ 8 $\mu\text{g/ml}$. This profile implies *ermB*-mediated ribosomal methylation. Only 11 (2.8%) of the total of 394 erythromycin-resistant isolates did not fit one of these two profiles.

Resistance rates (I + R) to other non-beta-lactam agents tested in this study were as follows: clindamycin, 9.2%; tetracycline, 16.6%; chloramphenicol, 8.3%; and TMP-SMX, 35.9%. Only one strain was resistant to rifampin (MIC > 4 $\mu\text{g/ml}$), and only one strain was intermediate to quinupristin-

TABLE 3. Recovery of *S. pneumoniae* strains with intermediate and high levels of resistance, by specimen source and patient characteristics, 1999–2000

Characteristic	Total no. (%) of isolates	No. (%) of isolates resistant to:										
		Penicillin		Ceftriaxone		Erythromycin		Tetracycline		TMP-SMX		Chloramphenicol, R ^a
		I	R	I	R	I	R	I	R	I	R	
Specimen source												
Upper respiratory tract	326 (21.3)	46 (14.1)	102 (31.3)	42 (12.9)	74 (22.7)	1 (0.3)	120 (36.8)	1 (0.3)	73 (22.4)	28 (8.6)	132 (40.5)	37 (11.4)
Lower respiratory tract	681 (44.5)	95 (14.0)	142 (20.9)	77 (11.3)	90 (13.2)	2 (0.3)	175 (25.7)	3 (0.4)	132 (19.4)	35 (5.1)	197 (28.9)	67 (9.8)
Blood	458 (29.9)	49 (10.7)	68 (14.9)	29 (6.3)	48 (10.5)	3 (0.7)	82 (17.9)	0 (0.0)	39 (8.5)	18 (3.9)	116 (25.3)	22 (4.8)
Cerebrospinal fluid/ normally sterile body fluid	33 (2.2)	2 (6.1)	8 (24.2)	5 (15.2)	4 (12.1)	0 (0.0)	7 (21.2)	0 (0.0)	1 (3.0)	2 (6.1)	7 (21.2)	1 (3.0)
Other	33 (2.2)	2 (6.1)	9 (27.3)	4 (12.1)	5 (15.2)	1 (3.0)	10 (30.3)	0 (0.0)	5 (15.2)	2 (6.1)	12 (36.4)	0 (0.0)
Age group												
0–5 yr	447 (29.2)	61 (13.7)	129 (28.9)	55 (12.3)	89 (19.9)	0 (0.0)	147 (32.9)	1 (0.2)	79 (17.7)	37 (8.3)	171 (38.3)	39 (8.7)
6–20 yr	87 (5.7)	9 (10.3)	22 (25.3)	8 (9.2)	15 (17.2)	0 (0.0)	28 (32.2)	2 (2.3)	20 (23.0)	2 (2.3)	30 (34.5)	7 (8.1)
21–64 yr	643 (42.0)	72 (11.2)	121 (18.8)	59 (9.2)	80 (12.4)	2 (0.3)	142 (22.1)	1 (0.2)	92 (14.3)	33 (5.1)	165 (25.7)	42 (6.5)
≥65 yr	339 (22.1)	48 (14.2)	54 (15.9)	31 (9.1)	35 (10.3)	4 (1.2)	73 (21.5)	0 (0.0)	57 (16.8)	13 (3.8)	90 (26.6)	37 (10.9)
Service												
Inpatient	860 (56.2)	110 (12.8)	168 (19.5)	82 (9.5)	110 (12.8)	5 (0.6)	203 (23.6)	1 (0.1)	130 (15.1)	47 (5.5)	232 (27.0)	68 (7.9)
Outpatient	665 (43.4)	82 (12.3)	160 (24.1)	73 (11.0)	110 (16.5)	2 (0.3)	190 (28.6)	3 (0.5)	118 (17.7)	38 (5.7)	229 (34.4)	58 (8.7)

^a No isolates showed intermediate resistance to chloramphenicol.

dalfopristin (MIC = 2 µg/ml). No vancomycin-resistant strains were detected. The highest vancomycin MIC was 0.5 µg/ml.

Testing of the antimicrobial activities of seven fluoroquinolones against *S. pneumoniae* resulted in the following overall rank order of activity: gemifloxacin > sitafloxacin > moxifloxacin > gatifloxacin > levofloxacin = ciprofloxacin > ofloxacin. NCCLS breakpoints currently exist for four of the fluoroquinolones tested in this study: ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin (17). Resistance (I + R) rates were noted to be 5.6, 0.7, 0.4, and 0.3%, respectively. Although there are no NCCLS-approved breakpoints for ciprofloxacin versus *S. pneumoniae*, Chen and colleagues suggested a ciprofloxacin MIC of ≥4 µg/ml as a criterion for fluoroquinolone resistance based on the association between isolates with a ciprofloxacin MIC of ≥4 µg/ml and mutations in the fluoroquinolone-resistance-determining region of *S. pneumoniae*, as well as the fact that a ciprofloxacin MIC of ≥4 µg/ml exceeds the usual peak achievable serum level of ciprofloxacin (2). Based on this definition, 1.4% (*n* = 21) of the current collection of pneumococci were resistant to ciprofloxacin. Also of note is that 6 of these 21 strains had penicillin MICs of ≥2 µg/ml, 6 had penicillin MICs of 0.25 to 1 µg/ml, and the remaining 9 strains were penicillin susceptible. All six with penicillin MICs of ≥2 µg/ml were also multiresistant, with between two and four additional resistances.

Two ketolides were tested, ABT773 (Abbott Pharmaceutical) and telithromycin (Aventis Pharmaceutical). ABT773 was consistently four- to eightfold more active than telithromycin (Table 1). In comparison to penicillin-susceptible strains, the MIC₉₀s of ABT773 and telithromycin increased slightly among penicillin-intermediate (0.06 and 0.12 µg/ml, respectively) and penicillin-resistant (0.25 and 0.5 µg/ml, respectively) strains. These values for penicillin-susceptible strains were 0.015 and 0.015 µg/ml, respectively. NCCLS breakpoints have not yet

been established for either of these agents; therefore, rates of resistance were not determined.

The MIC₅₀ of linezolid, an oxazolidinone, was 1 µg/ml, and the MIC₉₀ was 2 µg/ml. No differences were seen when isolates were categorized based on their penicillin susceptibility category. NCCLS breakpoints have not yet been established for this antimicrobial; however, the Food and Drug Administration-approved susceptibility breakpoint included in the package insert is ≤2 µg/ml. Based on this criterion, 100% of *S. pneumoniae* strains in this study were susceptible to linezolid.

Of this collection of isolates, 22.4% (*n* = 343) were found to be multiresistant (defined as intermediate or resistant to penicillin plus intermediate or resistant to at least two non-beta-lactam agents) (Table 3). Of the multiresistant strains, 36.4% (*n* = 125) were resistant to penicillin, erythromycin, and TMP-SMX, 28.6% (*n* = 98) were resistant to penicillin, erythromycin, chloramphenicol, tetracycline, and TMP-SMX, and 23.6% (*n* = 81) were resistant to penicillin, erythromycin, tetracycline, and TMP-SMX. The remaining 39 isolates (11.4%) exhibited other combinations of three or four resistances.

Table 4 indicates rates of antimicrobial resistance to selected antimicrobial agents sorted according to selected patient and specimen demographic characteristics. Of the *S. pneumoniae* isolates in this collection, 65.8% (*n* = 1,007) were obtained from respiratory specimens (44.5% lower respiratory, 21.3% upper respiratory); 32.1% (*n* = 491) were invasive isolates recovered from blood, cerebrospinal fluid, or other normally sterile body fluids; 45.4% of upper respiratory specimens, 34.9% of lower respiratory specimens, and 25.9% of invasive isolates were penicillin intermediate or high-level resistant.

Of the *S. pneumoniae* isolates collected from children 0 to 5 years of age, 42.5% (*n* = 190) were penicillin intermediate or high-level resistant, a level 7 to 10% higher than the penicillin resistance rate in any other age category. Rates of penicillin

TABLE 4. Resistance rates of selected antimicrobials by individual study center, 1999–2000

Medical center	Geographic location	No. of isolates	Resistance to:											
			Penicillin		Ceftriaxone		Erythromycin		Tetracycline		TMP-SMX		Chloramphenicol, % R	
			% I	% R	% I	% R	% I	% R	% I	% R	% I	% R		
Pathology Medical Laboratories	San Diego, Calif.	30	10.0	23.3	10.0	13.3	0.0	20.0	0.0	16.7	6.7	23.3	13.3	
UCLA Medical Center	Los Angeles, Calif.	51	5.9	15.7	9.8	5.9	0.0	21.6	0.0	15.7	5.9	19.6	3.9	
UCSF Medical Center	San Francisco, Calif.	52	9.6	23.1	19.2	9.6	0.0	30.8	0.0	19.2	3.9	32.7	11.5	
Veteran's Affairs Medical Center	Portland, Oreg.	22	22.7	31.8	13.6	22.7	0.0	40.9	0.0	50.0	0.0	50.0	27.3	
University of Washington	Seattle, Wash.	50	18.0	18.0	10.0	12.0	0.0	26.0	0.0	24.0	6.0	34.0	10.0	
Texas Children's Hospital	Houston, Tex.	55	20.0	38.2	20.0	21.8	0.0	49.1	0.0	27.3	9.1	50.9	12.7	
University of New Mexico	Albuquerque, N.Mex.	49	12.2	8.2	4.1	8.2	0.0	12.2	0.0	12.2	0.0	14.3	6.1	
University of Texas Southwestern Medical Center	Dallas, Tex.	44	11.4	15.9	2.3	13.6	0.0	27.3	0.0	15.9	9.1	25.0	9.1	
Denver Health Medical Center	Denver, Colo.	51	21.6	13.7	9.8	7.8	0.0	21.6	0.0	19.6	3.9	27.5	2.0	
ARUP Laboratories, Inc.	Salt Lake City, Utah	50	16.0	16.0	8.0	12.0	2.0	16.0	0.0	8.0	4.0	36.0	10.0	
Good Samaritan Medical Center	Phoenix, Ariz.	59	10.2	35.6	17.0	22.0	1.7	32.2	0.0	25.4	5.1	44.1	6.8	
Cleveland Clinic Foundation	Cleveland, Ohio	52	7.7	34.6	15.4	21.2	0.0	38.5	0.0	19.2	1.9	44.2	15.4	
Children's Hospital Wisconsin	Milwaukee, Wis.	53	11.3	32.1	15.1	20.8	0.0	34.0	0.0	16.7	7.6	41.5	9.4	
Henry Ford Hospital	Detroit, Mich.	58	8.6	5.2	0.0	5.2	0.0	10.3	0.0	8.6	3.5	15.5	5.2	
Mayo Clinic	Rochester, Minn.	49	8.2	16.3	10.2	10.2	2.0	30.6	0.0	10.2	10.2	22.5	4.1	
Clarian Health Methodist Hospital	Indianapolis, Ind.	56	10.7	19.6	17.9	8.9	0.0	23.2	0.0	10.7	5.4	23.2	7.1	
University of Iowa College of Medicine	Iowa City, Iowa	54	11.1	16.7	3.7	14.8	0.0	18.5	1.9	11.1	1.9	33.3	3.7	
Rush-Presbyterian St. Luke's Medical Center	Chicago, Ill.	41	14.6	12.2	7.3	7.3	0.0	17.1	0.0	7.3	2.4	24.4	7.3	
Evanston Northwestern Healthcare	Evanston, Ill.	37	13.5	10.8	5.4	8.1	2.7	24.3	0.0	8.1	10.8	21.6	2.7	
Dartmouth-Hitchcock Medical Center	Lebanon, N.H.	33	12.1	15.2	15.2	3.0	0.0	6.1	0.0	24.2	0.0	27.3	12.1	
Columbia Presbyterian Medical Center	New York, N.Y.	59	15.3	20.3	8.5	15.3	0.0	17.0	1.7	13.6	3.4	33.9	10.2	
Beth Israel Deaconess Medical Center	Boston, Mass.	31	6.5	19.4	6.5	16.1	0.0	12.9	0.0	12.9	3.2	16.1	9.7	
Temple University Hospital	Philadelphia, Pa.	52	19.2	7.7	9.6	3.9	1.9	13.5	0.0	9.6	5.8	13.5	1.9	
Hartford Hospital	Hartford, Conn.	50	12.0	10.0	10.0	6.0	2.0	16.0	0.0	12.0	6.0	22.0	2.0	
Children's Hospital National Medical Center	Washington, D.C.	20	5.0	35.0	10.0	25.0	0.0	45.0	0.0	20.0	20.0	40.0	5.0	
University Hospital SUNY Health Science Center	Syracuse, N.Y.	48	2.1	20.8	6.3	14.6	0.0	20.8	0.0	10.4	10.4	25.0	6.3	
Geisinger Medical Center	Danville, Pa.	51	15.7	33.3	5.9	31.4	0.0	39.2	0.0	27.5	3.9	37.3	19.6	
University of Rochester Medical Center	Rochester, N.Y.	50	18.0	22.0	12.0	18.0	0.0	28.0	0.0	24.0	4.0	32.0	14.0	
University of North Carolina Hospital	Chapel Hill, N.C.	41	9.8	56.1	17.1	39.0	0.0	53.7	2.4	22.0	9.8	53.7	7.3	
Dekalb General Hospital	Decatur, Ga.	59	17.0	32.2	15.3	23.7	0.0	30.5	1.7	18.6	5.1	39.0	5.1	
University of South Alabama Medical Center	Mobile, Ala.	49	10.2	16.3	4.1	12.2	2.0	28.6	0.0	8.2	8.2	28.6	6.1	
Mount Sinai Medical Center	Miami Beach, Fla.	21	19.1	28.6	9.5	19.1	0.0	33.3	0.0	14.3	4.8	28.6	14.3	
University of Louisville Hospital	Louisville, Ky.	54	13.0	18.5	7.4	13.0	0.0	24.1	0.0	13.0	7.4	22.2	7.4	

resistance among inpatients (32.3%, $n = 278$) versus outpatients (36.4%, $n = 242$) were similar.

Table 5 compares resistance rates of selected antimicrobials by individual study center. Each medical center contributed an average of 46 unique patient isolates (range, 20 to 59 isolates). The frequency with which resistant (I + R) strains were recovered varied considerably between individual study centers: penicillin, 13.8 to 65.9%; ceftriaxone, 5.2 to 56.1%; erythromycin, 6.1 to 53.7%; tetracycline, 7.3 to 50.0%; TMP-SMX, 14.3 to 63.4%; and chloramphenicol, 1.9 to 27.3%. Of the 33 centers, 19 had penicillin resistance rates (I + R) that were lower than the national rate of 34.2%; 14 had rates of penicillin resistance higher than the national rate. Compared by geographic region, penicillin resistance rates (I + R) were as follows: west (five centers), 33.2%; southwest (six centers), 37.3%; midwest (eight centers), 29.3%; northeast (9 centers), 32.2%; and southeast (five centers), 42.9%.

DISCUSSION

The results of this study indicate that rates of antimicrobial resistance among clinical isolates of *S. pneumoniae* in the United States continue to increase. The current overall national rate of penicillin resistance is 34.2%; 21.5% are high-level resistant (MICs ≥ 2 $\mu\text{g/ml}$). Among 329 isolates for which MICs were ≥ 2 $\mu\text{g/ml}$, the percentages of organisms for which MICs were 2 and 4 $\mu\text{g/ml}$ were 71.4 and 28.6%, respectively. No isolates for which MICs were ≥ 8 $\mu\text{g/ml}$ were recognized. When organisms were grouped according to penicillin susceptibility categories, rates of resistance to all beta-lactam agents increased in parallel with penicillin resistance. Penicillin resistance in *S. pneumoniae* is the result of alterations in cell wall penicillin-binding proteins (PBPs) (8, 23). All beta-lactam antimicrobials, to at least some extent, use essentially the same PBPs as their target of action; therefore, alterations in PBPs that cause resistance to penicillin will result in some degree of

TABLE 5. Comparison of three national antimicrobial resistance surveillance studies

Period	No. of medical centers	No. of isolates	Age distribution (%)					Source (%) ^b		Antimicrobial resistance (%) ^b						Multi-resistance (%) ^{c,f}		
			0-65 yr			≥65 yr		LRT	Blood/CSF/BF	Other	Penicillin ^c	Erythromycin ^c	Clindamycin ^c	Tetracycline ^a	TMP-SMX ^c		Chloramphenicol ^d	Ciprofloxin ^e
			0-5 yr	6-20 yr	21-64 yr	≥65 yr	URT											
1994-1995	30	1,527	32.9	6.9	36.5	23.4	18.0	41.6	40.1	0.1	23.6 (14.1, 9.5)	10.3 (0.1, 10.2)	Not tested	7.6 (0.1, 7.5)	26.8 (8.4, 18.4)	4.3	1.2	9.1
1997-1998	34	1,601	27.0	6.1	40.8	25.4	14.9	48.3	35.5	1.3	29.5 (17.4, 12.1)	19.2 (0.4, 18.9)	5.7 (0.1, 5.6)	13.2 (0.3, 12.9)	31.0 (10.7, 20.4)	7.2	1.6	16.0
1999-2000	33	1,531	29.2	5.7	42.0	22.1	21.3	44.5	32.1	2.2	34.2 (12.7, 21.5)	26.2 (0.5, 25.7)	19.2 (0.3, 8.9)	16.6 (0.3, 16.3)	35.9 (5.6, 30.3)	8.3	1.4	22.4

^a URT, upper respiratory tract; LRT, lower respiratory tract; CSF/BF, cerebrospinal fluid/normally sterile body fluid.

^b Intermediate plus high-level resistance; values in parentheses are intermediate and high-level resistance rates, respectively.

^c Overall resistance rates were significantly higher ($P < 0.001$) in 1999-2000 than in 1994-1995 for penicillin, erythromycin, clindamycin, tetracycline, TMP-SMX, chloramphenicol, and multiresistant isolates.

^d Only high-level resistance category, no intermediate resistance.

^e 1,523 isolates tested from 1994-1995; 1,596 isolates tested from 1997-1998; MIC of ≥ 4 $\mu\text{g/ml}$ used to determine resistance.

^f Multiresistance is defined as intermediate or high-level penicillin resistance plus intermediate or high-level resistance to at least two non-beta-lactam agents.

resistance to all beta-lactam antimicrobials. Indeed, an absolute linear relationship between penicillin MICs and MICs of eight other β -lactam antimicrobials has recently been described for a large collection ($n = 3,194$) of recent *S. pneumoniae* clinical isolates from the United States (1).

Resistance rates (I + R) among non-beta-lactam agents were the following: macrolides, 26%; clindamycin, 9.2%; tetracycline, 16.6%; chloramphenicol, 8.3%; and TMP-SMX, 35.9%. Rates of resistance to all of these non-beta-lactam antimicrobials were consistently higher among penicillin-intermediate and -resistant *S. pneumoniae* strains compared to penicillin-susceptible strains.

The phenotypic expression of macrolide resistance in this study indicated that the efflux and MLS_B phenotypes remain the dominant mechanisms of resistance among pneumococci in the United States. Organisms in the first group account for approximately 65% of macrolide-resistant isolates; MLS_B isolates account for approximately 32%. It is possible that other recently described macrolide resistance determinants are present in those rare strains that do not clearly fit into either of these two phenotypes (26).

Vancomycin and quinupristin-dalfopristin resistance was not detected, and only 0.1% of *S. pneumoniae* strains were resistant to rifampin. Newer agents such as the ketolides and linezolid were consistently active, even among penicillin-nonsusceptible *S. pneumoniae* isolates.

Multiresistance continues to grow as a problem among *S. pneumoniae* isolates in the United States. Overall, 22.4% of the *S. pneumoniae* isolates tested in this study were resistant to at least three different classes of antimicrobials; 28.6% of the multiresistant strains were resistant to five different antimicrobial classes: penicillin, erythromycin, chloramphenicol, tetracycline, and TMP-SMX.

Antimicrobial resistance rates were highest among middle ear fluid and sinus isolates, isolates recovered from children ≤ 5 years of age, and isolates from patients residing in the southeastern United States. Inpatient and outpatient resistance rates were comparable.

The modal MICs of ofloxacin, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, sitafloxacin, and gemifloxacin for the *S. pneumoniae* isolates characterized in this study were 2, 1, 1, 0.25, 0.12, 0.03, and 0.015 $\mu\text{g/ml}$, respectively. For 21 strains (1.4%), ciprofloxacin MICs were ≥ 4 $\mu\text{g/ml}$. For 11 of these isolates, the ciprofloxacin MICs were 4 $\mu\text{g/ml}$; the MICs of ofloxacin, levofloxacin, gatifloxacin, moxifloxacin, sitafloxacin, and gemifloxacin for these 11 isolates were 2 to 16, 1 to 8, 0.25 to 2, 0.12 to 1, 0.06 to 0.25, and 0.03 to 0.12 $\mu\text{g/ml}$, respectively. For five strains, MICs were as follows: ciprofloxacin, 8 $\mu\text{g/ml}$; ofloxacin, 4 to 16 $\mu\text{g/ml}$; levofloxacin, 2 to 8 $\mu\text{g/ml}$; gatifloxacin, 0.5 to 4 $\mu\text{g/ml}$; moxifloxacin, 0.25 to 2 $\mu\text{g/ml}$; sitafloxacin, 0.12 to 0.25 $\mu\text{g/ml}$; and gemifloxacin, 0.03 to 0.25 $\mu\text{g/ml}$. Two strains with ciprofloxacin MICs of 16 $\mu\text{g/ml}$ had ofloxacin MICs of 4 and 8 $\mu\text{g/ml}$, levofloxacin MICs of 2 and 4 $\mu\text{g/ml}$, gatifloxacin MICs of 1 $\mu\text{g/ml}$, moxifloxacin MICs of 0.25 $\mu\text{g/ml}$, sitafloxacin MICs of 0.25 $\mu\text{g/ml}$, and gemifloxacin MICs of 0.25 $\mu\text{g/ml}$. For one strain, MICs were as follows: ciprofloxacin, 32 $\mu\text{g/ml}$; ofloxacin, 32 $\mu\text{g/ml}$; levofloxacin, 16 $\mu\text{g/ml}$; gatifloxacin, 4 $\mu\text{g/ml}$; moxifloxacin, 2 $\mu\text{g/ml}$; sitafloxacin, 0.25 $\mu\text{g/ml}$; and gemifloxacin, 0.25 $\mu\text{g/ml}$. Finally, two strains with ciprofloxacin MICs of >64 $\mu\text{g/ml}$ also had the following MICs: ofloxacin,

>64 µg/ml; levofloxacin, 64 µg/ml; gatifloxacin, 16 µg/ml; moxifloxacin, 4 and 8 µg/ml; sitafloxacin, 2 µg/ml; and gemifloxacin, 2 µg/ml.

While overall resistance rates among fluoroquinolones remain at low levels, it is important to note that 6 of the 21 ciprofloxacin-resistant strains in this study were high-level penicillin resistant and multiresistant (resistant to erythromycin, tetracycline, chloramphenicol, or TMP-SMX). Four strains had ciprofloxacin MICs of 4 to 8 µg/ml, penicillin MICs of 2 µg/ml, and resistance to two to four additional antimicrobials; two strains had ciprofloxacin MICs of 16 µg/ml, penicillin MICs of 2 to 4 µg/ml, and resistance to two to three additional antimicrobials.

Resistance rates among individual medical centers varied widely. Penicillin resistance (I + R) ranged from 13.8% to 65.9%, and rates of resistance to non-beta-lactam agents varied in a similar manner. Those medical centers with lower rates of penicillin resistance also tended to have lower rates of resistance to other antimicrobial classes, and those centers with the highest rates of penicillin resistance were among the centers with the highest rates of resistance to non-beta-lactam agents.

It is of interest to compare the observations of this study with those of Whitney and colleagues, who recently described the results of a longitudinal population based survey of antimicrobial resistance among invasive isolates of *S. pneumoniae* from seven large United States metropolitan areas and the State of Connecticut (29). Data were presented for the years 1995 to 1998. Accepting that the period of our study, winter of 1999–2000, was roughly one year more recent than the latest acquisition year of their survey and accepting the fact that their study was much more restricted geographically than ours, there were striking similarities between rates of resistance observed in the two surveys among systemic isolates of *S. pneumoniae*. For example, resistance rates in our survey and the study conducted by Whitney et al. were 25.8 and 24% with penicillin, 17.5 and 17% with cefotaxime, 18.6 and 17% with erythromycin, 29.1 and 30% with TMP-SMX, 8.1 and 7% with tetracycline, and 4.7 and 3% with chloramphenicol, respectively.

Since the present study is a continuation of a longitudinal surveillance program including two previous national studies, it presents a unique opportunity for comparison of resistance rates from a common group of geographically distributed medical centers over a 5-year period. The number of medical centers in each study was between 30 and 34. Twenty-four medical centers were common to the 1994–1995 and 1997–1998 studies, 32 centers were common to the 1997–1998 and 1999–2000 studies, and 22 medical centers participated in all three studies. The numbers of isolates collected were comparable (1,527 to 1,601), and the isolates were collected during the exact same time period—the winter months between November 1 and April 30. All of the studies were similar with respect to the age distribution of the patients from whom isolates were obtained as well as the proportion of isolates obtained from each specimen source (Table 5).

Penicillin resistance (I + R) increased significantly during the past 5 years in the United States, from 23.6% in 1994–1995 to 34.2% in 1999–2000 (Table 5). Macrolide resistance increased from 10.3% in 1994–1995 to 26.2% in 1999–2000. Clindamycin resistance increased 3.5% between 1997–1998

and 1999–2000 to a current rate of 9.2%. (Clindamycin was not tested in 1994–1995.) Tetracycline resistance has more than doubled, increasing from 7.6% in 1994–1995 to 16.6% in 1999–2000. TMP-SMX resistance has increased 9.1% since 1994–1995, to a current rate of 35.9%. Chloramphenicol resistance doubled, from 4.3% to 8.3% during the past five winter seasons.

The increase in macrolide resistance is of particular interest. In view of the relationship between MICs and resistance mechanisms, i.e., erythromycin MICs of 1 to 32 µg/ml suggest efflux (*mefE*) while MICs of ≥ 64 µg/ml imply MLS_B as a result of ribosomal methylation (*ermB*), we were able to assess the contribution of these two resistance determinants to macrolide resistance over time. The relative percentage of macrolide-resistant strains with these two mechanisms did not change during the three studies we have conducted, i.e., 68.1 to 74.7% efflux phenotype in 1994–1995, 1997–1998, and 1999–2000, with 25.3 to 31.9% MLS_B phenotype during the same three periods.

In a recent survey of invasive pneumococcal isolates conducted over essentially the same period in the metropolitan Atlanta area, the relative percentage of efflux strains was found to have increased (7). Furthermore, there was a shift during the study period toward higher macrolide MICs with efflux-positive strains (7). Again, we have not observed this. The MIC_{50} s for efflux-positive isolates in our three surveys have all been 4 µg/ml; the MIC_{90} s for such isolates were 16 µg/ml in 1994–1995 and 8 µg/ml in both 1997–1998 and 1999–2000. The differences between our observations and those of Gay et al. (7) might be accounted for by the limited geographic area surveyed in their study and/or the fact that they reported results only for invasive isolates of *S. pneumoniae*.

Fluoroquinolone resistance with *S. pneumoniae* in the United States has remained stable during the past 5 years. Using a ciprofloxacin MIC of ≥ 4 µg/ml as a marker for fluoroquinolone resistance, resistance to ciprofloxacin in the United States has remained unchanged (i.e., 1.2 to 1.6%) during the last 5 years. Current overall fluoroquinolone resistance rates in Canada and the United States are remarkably similar (2). One difference, however, is that fluoroquinolone resistance rates in Canada appear to be changing rapidly, while our study indicates that resistance rates in the United States, as noted above, have remained essentially unchanged over the past 5 years.

Another important observation in this study is that the proportion of high-level penicillin resistant strains present in the United States now exceeds the proportion of penicillin-intermediate strains. Overall, 63% of penicillin-nonsusceptible strains in this study were high-level penicillin resistant, compared to 41% in 1997–1998 (6) and 40% in 1994–1995 (5). This is a startling increase in high-level penicillin resistance within just the past 2 years, but it is even more alarming compared to the resistance rates reported only 8 years ago. In 1991–1992, the overall national rate of penicillin resistance was 17.8%; only 2.6% of resistant isolates were high-level resistant (27). Of particular importance is the observation that the increased proportion of high-level penicillin-resistant isolates was noted in all three major specimen categories: upper respiratory tract, 68.9%; lower respiratory tract, 59.9%; and invasive isolates, 59.8%. While the clinical relevance of penicillin-resistant *S.*

pneumoniae in lower respiratory tract infections is debatable, there can be no doubt as to the clinical relevance and therapeutic challenge of penicillin resistance—particularly high-level resistance—among isolates causing systemic infections such as meningitis and perhaps other closed-space infections such as sinusitis or otitis media (12, 14, 19).

The rate of multiresistant *S. pneumoniae* has increased steadily: 9.1% in 1994–1995, 16.0% in 1997–1998, and 22.4% in 1999–2000. In this study, 65.5% of penicillin-resistant *S. pneumoniae* isolates were multiresistant. This also differs significantly from previous years. In 1997–1998, 54.0% of penicillin-nonsusceptible strains were multiresistant ($P < 0.0001$); in 1994–1995, 38.2% of penicillin-nonsusceptible strains were multiresistant ($P < 0.0001$). It is disconcerting to note that currently one-third of clinical isolates of *S. pneumoniae* are resistant to penicillin, of which ca. two-thirds are both high-level resistant as well as resistant to two or more non-beta-lactam antimicrobial classes.

One possible explanation for the increase in high-level penicillin resistance and multiresistance is the proliferation of a few major clones of penicillin-resistant or multiresistant strains in the United States. Two separate studies have characterized recent (1994–1995 and 1996–1997) clinical isolates of penicillin-resistant pneumococci at a molecular level to determine whether clonal relationships exist among these resistant organisms (3, 4). Both studies found that 9 to 10 major clones of penicillin-resistant *S. pneumoniae* exist in the United States, which together comprise 70 to 85% of the penicillin-resistant pneumococci in the United States (3, 4). The study by Corso and colleagues also determined that the most frequently occurring clone within the collection of 328 *S. pneumoniae* isolates that they characterized was the 23F clone from Spain, which is also a multiresistant clone (3). We speculate that the substantial increase in resistance rates during such a short period of time is suggestive of further proliferation of a few of these major clones of pneumococci, particularly high-level penicillin-resistant and multiresistant clones. Molecular characterization of the penicillin-resistant isolates from our study is under way and will hopefully provide additional insight into the epidemiology that surrounds the spread of antimicrobial-resistant *S. pneumoniae*. In conclusion, the problem of antimicrobial resistance with *S. pneumoniae* in the United States continues to grow.

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