ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1988, p. 180–185
0066-4804/88/020180-06$02.00/0

Vol. 32, No. 2

National Collaborative Study of the Prevalence of Antimicrobial Resistance among Clinical Isolates of *Haemophilus influenzae*

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A total of 2,811 clinical isolates of *Haemophilus influenzae* were obtained during 1986 from 30 medical centers and one nationwide private independent laboratory in the United States. Among these, 757 (26.9%) were type b strains. The overall rate of β-lactamase-mediated ampicillin resistance was 20.0%. Type b strains were approximately twice as likely as non-type b strains to produce β-lactamase (31.7 versus 15.6%). The MICs of 12 antimicrobial agents were determined for all isolates. Ampicillin resistance among strains that lacked β-lactamase activity was extremely uncommon (0.1%). Percentages of study isolates susceptible to cefamandole, cefaclor, cephalothin, and cephalexin were 98.7, 94.5, 87.3, and 43.3%, respectively. For 14 strains (0.5% of the total), chloramphenicol MICs were ≥8.0 μg, and thus the strains were considered resistant. All of these resistant strains produced chloramphenicol acetyltransferase. In addition, all 14 strains were resistant to tetracycline; 11 produced β-lactamase. The percentage of isolates susceptible to tetracycline was 97.7%. In contrast, erythromycin and sulfisoxazole were relatively inactive. The combination of erythromycin-sulfisoxazole (1/64) was more active than erythromycin alone but essentially equivalent in activity to sulfisoxazole alone. Finally, small numbers of clinical isolates of *H. influenzae* were resistant to trimethoprim-sulfamethoxazole and rifampin.

Antimicrobial resistance among clinical isolates of *Haemophilus influenzae* has become an increasingly prevalent problem (G. V. Doern, Antimicrob. Newsl. 5:28–34, 1986). In a national collaborative study conducted in 1984, 15.2% of a large number of strains of *H. influenzae* produced β-lactamase (6). The problem of ampicillin resistance is complicated by recent descriptions of clinical isolates of *H. influenzae* that are resistant to ampicillin by mechanisms other than the production of a TEM-type β-lactamase (10, 11, 14). In addition, chloramphenicol resistance has now been reported (2, 15), as has resistance to a variety of alternative agents commonly used to treat *Haemophilus* infections (Doern, Antimicrob. Newsl. 5:28–34). The intent of this investigation was to define systematically the prevalence of antimicrobial resistance among clinical isolates of *H. influenzae* in the United States. Rates of β-lactamase production and the activities of 12 antimicrobial agents were assessed. These agents included ampicillin, chloramphenicol, cefamandole, cefaclor, cephalothin, cephalexin, tetracycline, rifampin, erythromycin, sulfisoxazole, and the combinations erythromycin-sulfisoxazole and trimethoprim-sulfamethoxazole (TMP-SMX).

MATERIALS AND METHODS

Study centers. A total of 30 hospital-based microbiology laboratories and 1 national, private, independent laboratory participated in the study (Table 1).

Clinical isolates. A total of 2,811 clinical isolates of *H. influenzae* were obtained as part of this investigation in 1986 by the laboratories listed in Table 1. All isolates were recovered from different patients and were randomly selected for inclusion in the study. After being characterized in study center laboratories, isolates were subcultured to chocolate agar slants (GIBCO Diagnostics, Madison, Wis.), which were incubated overnight in a CO_2 atmosphere and then mailed, with selected patient demographic information, to one of two coordinating study centers for further characterization. The coordinating study centers were the Department of Clinical Microbiology, University of Massachusetts Medical Center, Worcester, and the Department of Pathology, University of Texas Health Science Center, San Antonio. Upon receipt in the coordinating study centers, growth from slants was transferred into 10% sterile skim milk and frozen at −70°C in 1-dram (ca. 3.7-ml) plastic freezer vials.

Isolate characterization. Frozen stock suspensions were thawed, and aliquots were subcultured to chocolate agar plates (GIBCO) which were incubated overnight at 35°C in 5 to 7% CO_2. Individual isolated colonies were then subcultured to a second chocolate agar plate which was incubated under identical conditions. Growth from the second plate was used for the following analyses. Organisms were identified as *H. influenzae* on the basis of the following characteristics: typical colony and Gram stain morphology (8), catalase production (7), hemin dependence on the basis of the porphyrin test (8), and lack of hemolysis when grown on tryptic soy agar plates containing 5% horse blood agar and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.). Isolates identified as *H. influenzae* were further examined for type b capsular antigen by a slide agglutination procedure (8) which uses *H. influenzae* type b antiserum (Burroughs Wellcome, Co., Research Triangle Park, N.C.).

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TABLE 1. Sources of *H. influenzae* isolates and prevalence of β-lactamase production

Study center[a]	No. of strains (% β-lactamase positive)	
	Type b	Non-type b
Northeast		
BVA	20 (10.0)	71 (8.5)
UCHC	59 (24.5)	41 (22.0)
CHB	24 (25.0)	62 (24.2)
NYUMC	17 (94.1)	75 (0.0)
LIJMC	53 (32.1)	50 (24.0)
TJH	13 (7.7)	74 (13.5)
SCHC	31 (22.6)	62 (22.6)
Southeast		
MCV	39 (33.3)	53 (18.9)
JCVA	2 (0.0)	79 (10.1)
VUMC	47 (23.4)	48 (29.2)
MUSC	27 (25.9)	58 (8.6)
GMH	14 (50.0)	78 (9.0)
BCH	42 (23.8)	57 (26.3)
Midwest		
OSUH	27 (14.8)	67 (10.5)
CCH	23 (26.1)	63 (19.1)
NUMH	12 (16.7)	49 (22.5)
EH	23 (60.9)	64 (15.6)
MCW	3 (0.0)	83 (14.5)
CHW	38 (34.2)	51 (15.7)
SPRMC	10 (40.0)	55 (9.1)
SLCH	39 (43.6)	33 (18.2)
Southwest		
HH	19 (47.4)	76 (8.6)
MCH	26 (26.9)	45 (28.9)
LMC	5 (40.0)	58 (17.2)
GSMC	34 (38.2)	60 (16.7)
West		
UCLA	10 (20.0)	73 (17.8)
LLUMC	10 (50.0)	82 (20.7)
IDMG	4 (50.0)	34 (17.7)
KPRL	31 (32.3)	44 (20.5)
COH	42 (40.5)	55 (29.1)
Nationwide		
MML	13 (7.7)	254 (10.6)
Totals	757 (31.7)	2,054 (15.6)

[a] BVA, Veterans Administration Hospital, Boston, Mass; UCHC, University of Connecticut Health Center, Farmington; CHB, Children's Hospital of Buffalo, Buffalo, N.Y.; NYUMC, New York University Medical Center, New York; LIJMC, Long Island Jewish Hillside Medical Center, New Hyde Park, N.Y.; TJH, Thomas Jefferson Hospital, Philadelphia, Pa.; SCHC, St. Christopher's Hospital for Children, Philadelphia, Pa.; MCV, Medical College of Virginia, Richmond; JCVA, Veterans Administration Medical Center, Johnson City, Tenn.; VUMC, Vanderbilt University Medical Center, Nashville, Tenn.; MUSC, Medical University of South Carolina, Charleston; GMH, Grady Memorial Hospital, Atlanta, Ga.; BCH, Children's Hospital, Birmingham, Ala.; OSUH, Ohio State University Hospitals, Columbus; CCH, Children's Hospital Medical Center, Cincinnati, Ohio; NUMH, Northwestern University Memorial Hospital, Chicago, Ill.; EH, Evanston Hospital, Evanston, Ill.; MCW, Medical College of Wisconsin, Milwaukee; CHW, Children's Hospital of Wisconsin, Milwaukee; SPRMC, St. Paul-Ramsey Medical Center, St. Paul, Minn.; SLCH, St. Louis Children's Hospital, St. Louis, Mo.; HH, Herman Hospital, Houston, Tex.; MCH, Medical Center Hospital, San Antonio, Tex.; LMC, Lovelace Medical Center, Albuquerque, N.Mex.; GSMC, Good Samaritan Medical Center, Phoenix, Ariz.; UCLA, University of California at Los Angeles Hospital, Los Angeles; LLUMC, Loma Linda University Medical Center, Loma Linda, Calif.; IDMG, Infectious Disease Medical Group, Oakland, Calif.; KPRL, Kaiser Permanente Regional Laboratory, Portland, Oreg.; COH, Children's Orthopedic Hospital, Seattle, Wash.; MML, Metpath Microbiology Laboratory, Teterboro, N.J.

TABLE 2. Ages of patients from whom *H. influenzae* was recovered

Age group	Total no. (%) of strains	No. of strains (% β-lactamase positive)	
		Type b	Non-type b
Not indicated	227 (8.1)	53 (32.1)	174 (16.7)
0–1 mo	44 (1.6)	5 (20.0)	39 (12.8)
2 mo–2 yr	824 (29.3)	424 (34.0)	400 (24.5)
>2–5 yr	233 (8.3)	106 (30.2)	127 (19.7)
>5–10 yr	120 (4.3)	27 (40.7)	93 (15.1)
>10–20 yr	172 (6.1)	11 (36.4)	161 (14.9)
>20–50 yr	507 (18.0)	54 (27.8)	453 (13.0)
>50 yr	684 (24.3)	77 (20.8)	607 (11.0)

In addition, β-lactamase production was assessed with nitrocefin-impregnated paper disks (Cefinase disks; BBL) (12). Selected isolates were tested for chloramphenicol acetyltransferase activity by the method of Azumen and coworkers (1). *H. influenzae* strains with known reactivities were used as test controls.

Antimicrobial susceptibility testing. MICs were determined with commercially prepared dehydrated microdilution susceptibility test plates (Sensititre, Inc., Salem, N.H.). The 12 antimicrobial agents were examined against all study isolates at the following ranges of concentrations: ampicillin, chloramphenicol, cephalothin, cephalexin, cefaclor, cefamandole, tetracycline, erythromycin, and sulfisoxazole, 0.008 to 256 μg/ml; erythromycin-sulfisoxazole, 0.008/0.5 to 16/1,024 μg/ml; TMP-SMX, 0.008/0.15 to 32/608 μg/ml; and rifampin, 0.008 to 128 μg/ml.

Microdilution test plates were inoculated (100 μl per well) with a suspension of test organism (1×10^5 to 5×10^5 CFU/ml) in *Haemophilus* Test Medium by using an automated inoculator (Sensititre). *Haemophilus* test medium consisted of Mueller-Hinton broth (GIBCO or Difco Laboratories, Detroit, Mich.), 15 μg of β-NAD (Sigma Chemical Co., St. Louis, Mo.) per ml, 15 μg of bovine hematin (Sigma) per ml, 5 mg of yeast extract (GIBCO or Scott Laboratories, Inc., Fiskeville, R.I.) per ml, 0.2 IU of thymidine phosphorylase (Burroughs Wellcome) per ml, 25 mg of magnesium per liter, and 50 mg of calcium per liter, at pH 7.3 (J. H. Jorgensen, J. S. Redding, L. A. Maher, and A. W. Howell, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C-219, p. 359). Microdilution test plates were incubated for 20 to 24 h at 35°C in ambient air, and wells were examined macroscop-

TABLE 3. Specimen types from which *H. influenzae* was recovered

Specimen type[a]	Total no. (%) of strains	No. of strains (% β-lactamase positive)	
		Type b	Non-type b
Not indicated	28 (1.0)	3 (66.7)	25 (12.0)
Blood	322 (11.5)	254 (33.1)	68 (19.1)
CSF	266 (9.5)	259 (34.0)	7 (0.0)
Body fluid	28 (1.0)	10 (50.0)	18 (5.6)
Sputum	970 (34.5)	103 (15.5)	867 (12.7)
TTA	101 (3.6)	12 (50.0)	89 (14.6)
Middle ear fluid	81 (2.9)	8 (12.5)	73 (26.0)
URT	414 (14.7)	44 (45.5)	370 (16.2)
Eye	395 (14.1)	33 (24.2)	362 (18.0)
Other	206 (7.3)	31 (32.3)	175 (21.1)

[a] CSF, Cerebrospinal fluid; TTA, transtracheal aspirate; URT, upper respiratory tract.

TABLE 4. MICs of selected beta-lactam antimicrobial agents for 561 β-lactamase-positive strains and 2,250 β-lactamase-negative strains of *H. influenzae*

Antimicrobial agent	β-Lactamase	No. of strains (cumulative %) for which MIC (μg/ml) was as follows:						
		<0.015	0.03	0.06	0.125	0.25	0.5	1.0
Ampicillin	Positive							
	Negative	1 (0.04)		11 (0.5)	67 (3.5)	752 (36.9)	1,238 (92.0)	110 (96.8)
Cephalexin	Positive							
	Negative		1 (0.04)	1 (0.1)			3 (0.2)	9 (0.6)
Cephalothin	Positive					2 (0.4)	6 (1.9)	26 (6.5)
	Negative				4 (0.2)	18 (1.0)	46 (3.0)	203 (12.0)
Cefaclor	Positive						3 (0.5)	25 (5.0)
	Negative	2 (0.1)	2 (0.2)		2 (0.3)	1 (0.3)	23 (1.3)	89 (5.3)
Cefamandole	Positive					5 (0.9)	42 (8.4)	194 (43.0)
	Negative		1 (0.04)	1 (0.1)	14 (0.7)	48 (2.8)	481 (24.2)	1,167 (76.1)

ically for evidence of growth. An MIC was defined as the lowest concentration of antimicrobial agent tested which inhibited growth. A strain of *H. influenzae* for which the MICs of the antimicrobial agents are known was used as a daily control.

RESULTS

A total of 2,811 clinical isolates of *H. influenzae* were examined (Table 1). Among these, 757 (26.9%) were encapsulated type b strains, and 2,054 (72.1%) were non-type b strains. The number of isolates contributed by each of the 30 hospital-based study centers varied from a low of 38 (Infectious Disease Medical Group) to a high of 103 (Long Island Jewish Hillside Medical Center), with a mean of 85 isolates per center. In addition, 267 isolates were contributed by Metpath Microbiology Laboratory, a nationwide, private, independent laboratory.

The largest number of type b strains were obtained from patients between the ages of 2 months and 2 years (Table 2). In contrast, non-type b strains were obtained most frequently from patients greater than 50 years of age. Type b strains were recovered most frequently from blood and cerebrospinal fluid, while sputum, upper respiratory tract, and eye cultures were the most common sources of non-type b strains (Table 3).

The overall rate of β-lactamase production among type b strains was 31.7%. In contrast, 15.6% of non-type b strains produced β-lactamase (Table 1). The overall prevalence of β-lactamase production among the 2,811 study isolates was thus 20.0%. In general, whether isolates were categorized by study center (Table 1), patient age (Table 2), or specimen source (Table 3), type b strains were more likely to produce β-lactamase than were non-type b strains.

The in vitro activities of five beta-lactam antimicrobial agents against the 2,811 study isolates are shown in Table 4. For all of the 561 β-lactamase-producing strains, ampicillin MICs were ≥2.0 μg/ml. Indeed, for most of these strains (i.e., 452 or 80.6%), ampicillin MICs were ≥16 μg/ml. For 2,179 of 2,250 isolates (96.8%) that lacked β-lactamase, ampicillin MICs were ≤1.0 μg/ml. For 69 β-lactamase-negative strains (8 type b and 61 non-type b strains), ampicillin MICs were 2.0 or 4.0 μg/ml. In addition, two non-type b strains for which MICs were 16 and 32 μg/ml demonstrated high-level ampicillin resistance despite the absence of β-lactamase activity.

Cephalexin, cephalothin, cefaclor, and cefamandole had variable activities against the study isolates (Table 4). In general, cefamandole was the most active agent, followed by cefaclor, cephalothin, and cephalexin. With these four antimicrobial agents, in vitro activity was not noticeably influenced by β-lactamase production. On the basis of the MIC interpretative standards of the National Committee for Clinical Laboratory Standards (NCCLS), i.e., MICs of ≤8.0 μg/ml indicate susceptibility (13), the overall percentages of study isolates susceptible to cefamandole, cefaclor, cepha-

TABLE 5. MICs of seven antimicrobial agents for 2,811 clinical isolates of *H. influenzae*

Antimicrobial agent	No. of strains (cumulative %) for which MIC (μg/ml) was as follows:						
	≤0.008	0.015	0.03	0.06	0.125	0.25	0.5
Chloramphenicol				2 (0.1)	11 (0.5)	80 (3.3)	1,828 (68.3)
Tetracycline					3 (0.1)	28 (1.1)	709 (26.3)
Erythromycin				2 (0.1)	3 (0.2)	18 (0.8)	6 (1.0)
Erythro-sulfa[a]	2 (0.1)	2 (0.2)	7 (0.4)	25 (1.3)	105 (5.0)	339 (17.1)	569 (37.3)
Sulfisoxazole		2 (0.1)			1 (0.1)		3 (0.2)
TMP-SMX[b]	58 (2.1)	134 (6.8)	694 (31.5)	1,386 (80.8)	463 (97.3)	43 (98.8)	8 (99.1)
Rifampin	2 (0.1)	4 (0.2)	1 (0.3)	11 (0.6)	244 (9.3)	1,523 (63.5)	1,005 (99.3)

[a] Erythro-sulfa, Erythromycin-sulfisoxazole. This combination was tested at a constant ratio of 1 part of erythromycin to 64 parts of sulfisoxazole; the concentration indicated is the concentration of erythromycin.

[b] This combination was tested at a constant ratio of 1 part of trimethoprim to 19 parts of sulfamethoxazole; the concentration indicated is the concentration of trimethoprim.

TABLE 4—*Continued*

No. of strains (cumulative %) for which MIC (μg/ml) was as follows:								
2.0	4.0	8.0	16	32	64	128	256	>256
17 (3.0)	33 (8.9)	59 (19.4)	93 (36.0)	142 (61.3)	106 (80.2)	66 (92.0)	26 (96.6)	19 (100)
65 (99.7)	4 (99.9)		1 (99.9)	1 (100)				
8 (1.4)	25 (5.9)	280 (55.8)	130 (79.0)	64 (90.4)	51 (99.5)	3 (100)		
40 (2.4)	71 (5.6)	778 (40.1)	708 (71.6)	328 (86.2)	239 (96.8)	35 (98.4)	19 (99.2)	18 (100)
104 (25.0)	117 (45.9)	202 (81.9)	77 (95.6)	24 (99.5)	3 (100)			
568 (37.3)	480 (58.6)	677 (88.7)	220 (98.5)	32 (99.9)	2 (100)			
115 (25.5)	243 (68.8)	128 (91.6)	29 (96.8)	6 (97.9)	7 (99.1)	5 (100)		
571 (30.6)	1,094 (79.3)	358 (95.2)	74 (98.5)	25 (99.6)	7 (99.9)	1 (99.9)	1 (100)	
238 (85.4)	54 (95.0)	22 (98.9)	5 (99.8)		1 (100)			
419 (95.7)	57 (97.2)	32 (98.7)	22 (99.6)	6 (99.9)	2 (100)			

lothin, and cephalexin were 98.7, 94.5, 87.3, and 43.3%, respectively.

The MICs of chloramphenicol, tetracycline, erythromycin, erythromycin-sulfisoxazole (1/64), sulfisoxazole, TMP-SMX (1/19), and rifampin are shown in Table 5. No differences were observed when the results obtained with these seven agents against β-lactamase-producing strains were compared with those obtained against strains that lacked β-lactamase. Chloramphenicol MICs for 2,797 isolates (99.5% of the total) were ≤4.0 μg/ml. For the remaining 14 strains (0.5% of the total; 2 type b and 12 non-type b strains), chloramphenicol MICs were ≥8.0 μg/ml; all of these strains produced chloramphenicol acetyltransferase. Eleven strains (2 type b, and 9 non-type b stains) were β-lactamase positive. For all 14 strains, tetracycline MICs were ≥8.0 μg/ml. From the 2,797 isolates for which chloramphenicol MICs were ≤4.0 μg/ml, 50 strains were selected for the assessment of chloramphenicol acetyltransferase production. These included 3 strains for which the MIC was 4.0 μg/ml, 1 strain for which the MIC was 2.0 μg/ml, 20 strains for which the MIC was 1.0 μg/ml, 20 strains for which the MIC was 0.5 μg/ml, and 6 strains for which the MIC was 0.25 μg/ml. None produced chloramphenicol acetyltransferase. For 50 of 2,797 isolates (1.8%) for which chloramphenicol MICs were ≤4.0 μg/ml, tetracycline MICs were ≥8.0 μg/ml.

A total of 2,747 study isolates (97.7% of the total) were susceptible to tetracycline on the basis of the NCCLS guideline, i.e., MICs of ≤4.0 μg/ml (Table 5) (13). Of 64 strains for which tetracycline MICs were ≥8.0 μg/ml (20 type b and 44 non-type b strains), 23 (35.9%; 5 type b and 18 non-type b strains) produced β-lactamase.

Erythromycin was relatively inactive (Table 5). Only 29 strains (1.0%) were susceptible on the basis of the criterion of the NCCLS, i.e., MICs of ≤0.5 μg/ml (13). Similarly, sulfisoxazole was, in general, inhibitory only at high concentrations. The combination of erythromycin and sulfisoxazole was considerably more active than erythromycin alone but essentially equivalent to sulfisoxazole alone. Unfortunately, insufficient information exists upon which to base calculations of a percent susceptible value for the combination erythromycin-sulfisoxazole.

Among 2,811 study isolates, 25 (0.9%; 5 type b and 20 non-type b isolates) were not susceptible to TMP-SMX on the basis of the NCCLS definition of susceptibility to TMP-SMX, i.e., MICs of ≤0.5/9.5 μg/ml (Table 5) (13). Of these 25 strains, 17 (2 type b and 15 non-type b strains) were also β-lactamase positive. For 3 isolates (all β-lactamase producing and non-type b) among the 25 strains that were not susceptible to TMP-SMX, chloramphenicol and tetracycline MICs were ≥8.0 μg/ml. For these three strains, the actual TMP-SMX MICs were 2.0/38, 4.0/76, and 4.0/76 μg/ml. In addition, for one β-lactamase-negative, non-type b strain of *H. influenzae* which was not susceptible to TMP-SMX, the tetracycline MIC was 16 μg/ml. The strain was susceptible to chloramphenicol (MIC, 1.0 μg/ml).The TMP-SMX MIC for this strain was 16/304 μg/ml.

Finally, for 9 of 2,811 study isolates (0.7%), rifampin MICs were ≥4 μg/ml; thus, these 9 strains were not susceptible to

TABLE 5—*Continued*

No. of strains (cumulative %) for which MIC (μg/ml) was as follows:								
1.0	2.0	4.0	8.0	16	32	64	128	>128
872 (99.4)	1 (99.4)	3 (99.5)	8 (99.8)	6 (100)				
1,869 (92.8)	136 (97.7)	2 (97.7)	8 (98.0)	47 (99.7)	9 (100)			
55 (3.0)	179 (9.4)	1,113 (49.0)	1,258 (93.7)	176 (99.9)	1 (100)			
723 (63.0)	786 (91.0)	252 (99.9)	1 (100)					
4 (0.4)	23 (1.2)	37 (2.5)	158 (8.1)	416 (22.9)	567 (43.1)	670 (66.9)	420 (81.9)	510 (100)
1 (99.2)	4 (99.3)	12 (99.7)	4 (99.9)	4 (100)				
11 (99.6)	1 (99.7)	1 (99.7)	2 (99.8)	1 (99.8)		1 (99.9)	2 (99.9)	2 (100)

this agent on the basis of the NCCLS criterion (Table 5) (13). All nine strains were non-type b, β-lactamase negative, and susceptible to ampicillin, chloramphenicol, tetracycline, and TMP-SMX.

DISCUSSION

The results of this investigation suggest that the prevalence of β-lactamase-mediated ampicillin resistance among clinical isolates of *H. influenzae* is increasing in the United States. In a nationwide surveillance study conducted in 1984, 21.0% of 1,156 type b strains and 12.1% of 2,200 non-type b strains produced β-lactamase (6). The present investigation with isolates obtained in 1986 showed that 31.7% of 757 type b strains and 15.6% of 2,054 non-type b strains were β-lactamase positive. Two observations made in the 1984 study were corroborated by the results of the present investigation. First, the overall rates of β-lactamase production were highest among *H. influenzae* isolates from patients in certain age groups (i.e., ≤5 years of age) and among isolates from specimens representative of systemic *Haemophilus* infections (i.e., blood and cerebrospinal fluid). This observation was largely a result of the greater numbers of type b strains recovered from younger patients and from systemic sites. Second, it was not possible to predict with certainty the prevalence of β-lactamase-positive *H. influenzae* for a given geographic area, because of the absence of any observed geographic clustering of β-lactamase-producing strains. As a result, predictions of rates of β-lactamase production must be based on the experiences of individual medical centers.

It is very likely that the β-lactamase-positive strains of *H. influenzae* recovered in the present investigation produced a TEM-1-type β-lactamase. This assumption is based on two observations. First, the vast majority of β-lactamase-producing strains of *H. influenzae* possess this enzyme (Doern, Antimicrob. Newsl. 5:28–34). Second, the nitrocefin disk test used to detect β-lactamase activity in the present study does not yield positive results with strains that possess the ROB-type β-lactamase (10), the only other β-lactamase described for *H. influenzae*.

If this is true, it appears that high-level ampicillin resistance among *H. influenzae* strains that lack the TEM-type β-lactamase is extremely uncommon in the United States. For only 2 isolates (both non-type b) from among 2,250 β-lactamase-negative strains characterized in this study were ampicillin MICs ≥16 μg/ml. In addition, for four β-lactamase-negative isolates (all non-type b), ampicillin MICs were 4.0 μg/ml, and thus the isolates would be considered resistant on the basis of the criteria of the NCCLS (13). Of interest, however, is the observation that for 65 isolates which lacked β-lactamase, the ampicillin MIC was 2.0 μg/ml. These isolates might be considered moderately susceptible or even resistant to ampicillin despite the recommendations of the NCCLS (13), which suggests that *H. influenzae* strains for which ampicillin MICs are ≤2.0 μg/ml be considered susceptible (4). We base this assertion on the observation that for 17 β-lactamase-positive strains in the present study, the ampicillin MIC was 2.0 μg/ml. If *H. influenzae* strains for which ampicillin MICs are ≥2.0 μg/ml were considered resistant, then the overall rate of ampicillin resistance among β-lactamase-negative isolates would be 3.2%.

Four cephalosporin antimicrobial agents were examined. Their activities against *H. influenzae* were in the following order: cefamandole > cefaclor ≥ cephalothin > cephalexin. Only 36 strains (1.3% of the total) were not susceptible to cefamandole. In contrast, 155 (5.5% of the total) and 358 (12.7% of the total) strains were not susceptible to cefaclor and cephalothin, respectively. Cephalexin had limited activity against *H. influenzae*. Isolates were considered susceptible to these four cephalosporins if MICs were ≤8.0 μg/ml (13). Cephalosporin activity did not seem to be influenced by β-lactamase activity, that is, the MICs of a given agent were similar for β-lactamase-positive and -negative strains.

Chloramphenicol resistance appears to be relatively uncommon in the United States. For 14 strains (0.5% of the total), chloramphenicol MICs were ≥8.0 μg/ml, and thus these strains were considered resistant (5). All of these strains produced chloramphenicol acetyltransferase, and all were resistant to tetracycline. Two were type b strains; both produced β-lactamase. Of the 12 non-type b, chloramphenicol-resistant strains, 9 produced β-lactamase.

Tetracycline was also highly active against most study isolates. Among all strains tested, 97.7% were determined to be susceptible to tetracycline based on the NCCLS-recommended MIC breakpoint of ≤4.0 μg/ml (13). In contrast, both erythromycin and sulfisoxazole had limited activities against the *H. influenzae* strains examined in this investigation. Interestingly, erythromycin-sulfisoxazole, a combination frequently used to treat localized non-life-threatening *Haemophilus* infections in children, was considerably more active than erythromycin alone but equivalent in activity to sulfisoxazole alone. Clinical interpretation of MICs obtained with this combination, however, is difficult. For this investigation, a ratio of 1 part of erythromycin to 64 parts of sulfisoxazole was used to determine MICs (P. N. Whitley and S. I. Pelton, Program Abstr. 21st Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 11, 1981). For these in vitro observations of inhibitory activity to have clinical relevance, a ratio of erythromycin to sulfisoxazole of approximately 1:64 would have to be achieved in vivo when this combination is used to treat *Haemophilus* infections. At least with respect to otitis media, the ratio of erythromycin to sulfisoxazole obtained in middle ear fluid appears to be much lower (9).

While TMP-SMX was highly active against most study isolates, for 25 strains (0.9% of the total) MICs were ≥1.0/19 μg/ml, and thus these strains were not considered susceptible by NCCLS criteria (13). Notable were 3 non-type b strains among these 25 that also demonstrated resistance to chloramphenicol and tetracycline, as well as β-lactamase-mediated ampicillin resistance. These strains seem similar to the multiply resistant strains of *H. influenzae* recently reported in Barcelona, Spain (3). Finally, the results of the present investigation clearly documented the existence of at least small numbers of clinical isolates of *H. influenzae* with high-level resistance to rifampin.

In conclusion, the results of this extensive national surveillance study of antimicrobial resistance among clinical isolates of *H. influenzae* revealed the following. (i) The prevalence of β-lactamase-mediated ampicillin resistance continues to increase with both type b and non-type b strains. The rate of β-lactamase-mediated ampicillin resistance among type b strains (i.e., 31.7%) indicates that the use of ampicillin alone for the empiric therapy of systemic *Haemophilus* infections is inappropriate. (ii) *H. influenzae* strains that are resistant to ampicillin by mechanisms other than the production of TEM-type β-lactamase are extremely uncommon. (iii) Chloramphenicol resistance is also unusual; however, when it occurs, it is invariably the result of chloramphenicol acetyltransferase production and is associated with β-lactamase

production and tetracycline resistance. (iv) Resistance to TMP-SMX and rifampin, two antimicrobial agents previously considered nearly uniformly active against *H. influenzae*, was clearly documented. Fortunately, resistance to these agents remains uncommon; however, in the case of TMP-SMX it may co-occur with β-lactamase-mediated ampicillin resistance and resistance to chloramphenicol and tetracycline. (v) Cephalexin and erythromycin have no value in the treatment of infections caused by *H. influenzae*.

ACKNOWLEDGMENTS

We thank the following for providing study isolates: Stephen Brecher (Boston Veterans Administration Hospital), Raymond W. Ryan (University of Connecticut Health Center), Leonard J. LaScolea, Jr. (Children's Hospital of Buffalo), Philip M. Tierno, Jr. (New York University Medical Center), Henry Isenberg (Long Island Jewish Hillside Medical Center), Donald Jungkind (Thomas Jefferson Hospital), Karin L. McGowan (St. Christopher's Hospital for Children), Harry P. Dalton (Medical College of Virginia), Salvador Alvarez (Johnson City Veterans Administration Medical Center), Charles W. Stratton (Vanderbilt University Medical Center), Edward R. Bannister (Medical University of South Carolina), John E. McGowan, Jr. (Grady Memorial Hospital), Barry M. Gray (Birmingham Children's Hospital), Leona W. Ayers (Ohio State University Hospitals), Mary J. R. Gilchrist (Cincinnati Children's Hospital), Grant O. Westenfelder (Evanston Hospital), Silas G. Farmer (Medical College of Wisconsin), W. Michael Dunn (Children's Hospital of Wisconsin), Bert F. Woolfrey (St. Paul-Ramsey Medical Center), Gregory Storch (St. Louis Children's Hospital), Eugene W. Rypka (Lovelace Medical Center), Michael A. Saubolle (Good Samaritan Medical Center), David Bruckner (University of California at Los Angeles Hospital), John E. Lewis (Loma Linda University Medical Center), Patrick Joseph (Infectious Disease Medical Group), Ronald N. Jones (Kaiser Permanente Regional Laboratories), Arnold L. Smith (Children's Orthopedic Hospital), Karen Mills (Herman Hospital), and Candice Miller (Metpath Microbiology Laboratory). We also appreciate the secretarial assistance of Karen Spiewak.

This study was supported by a grant from Eli Lilly & Co., Indianapolis, Ind.

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