

Antibiotic Resistance among Clinical Isolates of *Haemophilus influenzae* in the United States in 1994 and 1995 and Detection of β -Lactamase-Positive Strains Resistant to Amoxicillin-Clavulanate: Results of a National Multicenter Surveillance Study

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A total of 1,537 clinical isolates of *Haemophilus influenzae* were recovered in 30 U.S. medical center laboratories between 1 November 1994 and 30 April 1995 and were characterized in a central laboratory with respect to serotype and β -lactamase production and the in vitro activities of 15 oral antimicrobial agents. Overall, 36.4% of the isolates were found to produce β -lactamase. The rank order of activity of six cephalosporins on the basis of MICs was cefixime > cefpodoxime > cefuroxime > loracarbef \geq cefaclor > cefprozil. On the basis of current National Committee for Clinical Laboratory Standards (NCCLS) breakpoints, the overall percentages of isolates found to be resistant or intermediate to these agents were as follows: 0.1, 0.3, 6.4, 16.3, 18.3, and 29.8, respectively (National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. M7-A4, 1995). Azithromycin was, on a weight basis, the most potent of the macrolides tested in this study, followed by erythromycin and then clarithromycin. Azithromycin was typically fourfold more active than erythromycin, which was, in turn, slightly more active than clarithromycin. However, when compared on the basis of the frequency of resistance determined by using current NCCLS breakpoints, there was essentially no difference between azithromycin and clarithromycin, i.e., 0.5 and 1.9%, respectively ($P = 0.086$). Interpretive breakpoints for erythromycin MIC tests versus *H. influenzae* have not been developed. Resistance to other non- β -lactam agents was variable, as follows: trimethoprim-sulfamethoxazole, 9.0%; chloramphenicol, 0.2%; tetracycline, 1.3%; and rifampin, 0.3%. Two conspicuous findings in this study were the identification of 39 strains of *H. influenzae* that were β -lactamase negative but ampicillin intermediate or resistant (BLNAR) and, even more surprisingly, 17 β -lactamase-positive isolates that were resistant to amoxicillin-clavulanate (BLPACR). Strains of *H. influenzae* in the first group have heretofore been very uncommon; organisms in the second group have not previously been described in the literature. The percentages of all study isolates comprised of BLNAR and BLPACR organisms were 2.5 and 1.1, respectively. Overall resistance to ampicillin was thus 38.9%, and that to amoxicillin-clavulanate was 4.5%.

Haemophilus influenzae is recognized as a frequent cause of a variety of infections among outpatients, including acute otitis media, sinusitis, acute purulent exacerbation of chronic bronchitis, and pneumonia. These infections are usually caused by non-type b strains of *H. influenzae*. Life-threatening invasive infections more commonly caused by encapsulated type b strains have largely disappeared, at least in circumstances and areas in which the protein-conjugated type b capsular polysaccharide vaccine has been used (3). Since localized infections such as those caused by non-type b *H. influenzae* isolates are often treated empirically, a knowledge of antibiotic resistance determined on the basis of systematic surveillance studies is essential.

Since the first description of β -lactamase-mediated ampicillin resistance in *H. influenzae* in the United States in 1974 (15, 27), this problem has become increasingly more prevalent (1,

6–9, 11, 12, 23). The last systematic U.S. national surveillance study conducted in 1993 revealed a prevalence of 33% β -lactamase production among non-type b *H. influenzae* isolates (23). In addition, resistance to oral cephalosporins, macrolides, and other orally administered agents such as trimethoprim-sulfamethoxazole (TMP-SMX), chloramphenicol, and tetracycline has been described (1, 8, 9, 12). In 1980, the problem of antibiotic resistance among *H. influenzae* isolates was complicated further by the description of β -lactamase-negative strains which were resistant to ampicillin by some other mechanism (2, 17), perhaps elaboration of altered penicillin-binding proteins (5, 16, 19, 22). These strains, referred to as β -lactamase negative but ampicillin intermediate or resistant (BLNAR) have fortunately remained uncommon, at least in the United States and Canada (1, 8, 12, 25, 26).

The intent of the current investigation was to define the contemporary levels of resistance to 15 oral antimicrobial agents for a large number of isolates of *H. influenzae* obtained from patients with a variety of community-acquired infections in 30 U.S. medical centers. This may be considered a point prevalence study since all isolates were collected during the

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TABLE 1. Prevalence of β -lactamase production among clinical isolates of *H. influenzae* recovered from outpatients at 30 different U.S. medical centers between 1 November 1994 and 30 April 1995

Medical center	City	Total no. of isolates	% of isolates β -lactamase positive
Children's Hospital	Boston, Mass.	42	31.0
University of Massachusetts Medical Center	Worcester, Mass.	76	23.7
Hartford Hospital	Hartford, Conn.	68	38.2
SUNY Medical Center	Syracuse, N.Y.	39	33.3
Strong Memorial Hospital	Rochester, N.Y.	58	27.6
Columbia-Presbyterian Hospital	New York, N.Y.	61	29.5
Temple University Medical Center	Philadelphia, Pa.	47	17.0
Geisinger Medical Center	Danville, Pa.	56	35.7
National Children's Hospital	Washington, D.C.	13	23.1
University of North Carolina Medical Center	Chapel Hill, N.C.	61	45.9
DeKalb General Hospital	Decatur, Ga.	58	34.5
Mt. Sinai Hospital	Miami, Fla.	51	29.4
University of South Alabama Medical Center	Mobile, Ala.	61	42.6
Cleveland Clinic	Cleveland, Ohio	57	28.1
Henry Ford Hospital	Detroit, Mich.	63	68.3
Methodist Hospital	Indianapolis, Ind.	64	37.5
Rush Presbyterian Medical Center	Chicago, Ill.	44	45.5
Evanston Hospital	Evanston, Ill.	45	44.4
Children's Hospital	Milwaukee, Wis.	50	26.0
Mayo Clinic	Rochester, Minn.	45	46.7
Jewish Hospital	St. Louis, Mo.	67	29.9
University of Texas Southwestern Medical Center	Dallas, Tex.	38	36.8
Texas Children's Hospital	Houston, Tex.	28	50.0
Denver General Hospital	Denver, Colo.	32	31.3
Primary Children's Hospital	Salt Lake City, Utah	61	54.1
Good Samaritan Hospital	Phoenix, Ariz.	64	32.8
Cedar's Sinai Hospital	Los Angeles, Calif.	16	56.3
Stanford University Medical Center	Palo Alto, Calif.	54	18.5
Kaiser Medical Center	Portland, Oreg.	62	35.5
Children's Hospital	Seattle, Wash.	55	45.5

relatively narrow 6-month time frame of 1 November 1994 through 30 April 1995. All analyses were performed in a central laboratory.

MATERIALS AND METHODS

Bacterial isolates and identification. Between 1 November 1994 and 30 April 1995, a total of 1,537 unique patient isolates of *H. influenzae* were obtained from various specimen sources from outpatients in 30 different U.S. medical centers. Isolates were collected consecutively and were transported to the University of Massachusetts Medical Center on rayon swabs immersed in 12 ml of Aimes semisolid transport medium containing charcoal. Stock cultures were prepared by using an absorbent bead system (ProLab Diagnostics, Austin, Tex.), and organisms were stored at -70°C until further use. All organisms were subcultured twice on chocolate agar prior to further characterization. Isolates were confirmed as *H. influenzae* by using conventional criteria (4), and serotyping was performed by a slide agglutination procedure with type b antiserum (Murex Diagnostics, Inc., Atlanta, Ga.).

Susceptibility studies. MICs were determined as described by the National Committee for Clinical Laboratory Standard (NCCLS) (21) by a broth microdilution procedure (100- μl total volume per well; final inoculum concentration, ca. 5×10^5 CFU/ml), with the trays incubated at 35°C in ambient air for 22 to 24 h prior to determining the results. *Haemophilus* Test Medium (HTM) broth was used for MIC determinations with *H. influenzae* (13). Fifteen antibiotics obtained from their respective manufacturers as laboratory-grade powders were each tested at 12 different concentrations in an attempt to limit the number of off-scale results. The antibiotics included ampicillin, amoxicillin-clavulanate (A-C; 2:1), cefaclor, loracarbef, cefprozil, cefuroxime, cefixime, cefpodoxime, erythromycin, azithromycin, clarithromycin, TMP-SMX (1:19), chloramphenicol, tetracycline, and rifampin. *H. influenzae* ATCC 49247, ATCC 49766, and ATCC 10211 were used as controls. β -Lactamase production was assessed for all isolates by the nitrocefin disk assay (Cefinase; Becton-Dickinson Microbiology Systems, Cockeysville, Md.) (20).

RESULTS

A total of 1,537 isolates of *Haemophilus influenzae* were characterized in this investigation (Table 1). A range of from

13 to 76 isolates were contributed by each of the 30 participating medical center laboratories (Table 1). Among these, 560 (36.4%) produced β -lactamase. The proportion of isolates found to produce β -lactamase in individual centers varied from 17.0 to 68.3%. Only 17 isolates (1.2%) among a total of 1,469 strains characterized produced type b capsular antigen.

Males yielded 55.4% of isolates; there was no significant difference in β -lactamase production between isolates from males and females (Table 2). Table 2 also depicts the distribution of test strains by patient age and specimen type. Isolates were most commonly obtained in specimens from the lower respiratory tract. β -Lactamase production was most often found among organisms recovered from middle ear fluid, sinus aspirates, and conjunctival swab specimens, specimens usually representative of *Haemophilus* infections in children. Consistent with this observation was the finding that isolates from children ages 0 to 5 years had the highest frequency of β -lactamase production (i.e., 45.8%). Among middle ear fluid, sinus aspirate, and conjunctival swab specimens, 72.5% were obtained from patients 0 to 5 years old. By contrast, sputum specimens yielded isolates which were β -lactamase positive 31.5% of the time; 78.1% of isolates in sputum specimens came from individuals ≥ 21 years old. One final observation regarding the source of isolates deserves mention. Among a total of 33 isolates obtained on blood culture, 21 (63.6%) were recovered from adults ≥ 21 years of age, 14 (42.4%) were from patients ≥ 50 years old, and 8 (24.2%) were obtained from children ≤ 5 years old.

Results of MIC determinations with 15 antimicrobial agents are presented in Table 3. The rank order of activity of the six cephalosporins examined in this study was cefixime > cefpo-

TABLE 2. Source of clinical isolates of *H. influenzae* by patient sex, age, and specimen

Characteristic and description	Total no. (%) of isolates	% of isolates β -lactamase positive	P value ^a
Gender			
Male	847 (55.4)	35.7	NS
Female	683 (44.6)	37.2	
Age group (yr)			
0-5	428 (28.0)	45.8	NS
6-10	74 (4.8)	32.4	
11-20	98 (6.4)	34.7	NS
21-50	357 (23.4)	32.5	≤ 0.001
≥ 51	570 (37.3)	32.1	≤ 0.001
Specimen type			
Middle ear fluid	78 (5.1)	46.1	NS
Sinus aspirate	72 (4.7)	56.9	
Conjunctival swab	232 (15.2)	51.7	NS
Lower respiratory tract	1064 (69.5)	31.5	0.008
Blood	34 (2.2)	26.5	NS
Cerebrospinal fluid	3 (0.2)	0.0	NS
Other	47 (3.1)	31.9	NS

^a Chi-square analysis; in all cases, comparisons were made to the first entry in a category. NS, not significant.

doxime > cefuroxime > loracarbef \geq cefaclor > cefprozil. On the basis of current NCCLS breakpoints, the percentages of isolates found to be resistant to these agents were 0.1, 0.3, 1.5, 8.1, 8.0, and 14.6, respectively (21). The percentages of strains noted to be intermediate to cefuroxime, loracarbef, cefaclor, and cefprozil were 4.9, 8.2, 10.3, and 15.2, respectively. Interpretive criteria for an intermediate category have not been defined for cefixime or cefpodoxime (21). On the basis of a comparison of the MICs at which 50% of isolates are inhibited (MIC₅₀s), MIC₉₀s, and geometric mean MICs plus resistance rates, it appeared that the activities of loracarbef, cefaclor, and cefprozil against β -lactamase-positive strains were slightly diminished compared with those against β -lactamase-negative strains. β -Lactamase production did not influence the activity of cefpodoxime, cefixime, or cefuroxime.

The rank order of activity on a weight basis with the macrolide agents was azithromycin > erythromycin \geq clarithromycin. MIC interpretive breakpoints have been defined by NCCLS only for azithromycin and clarithromycin (21). By using these breakpoints, there was only a small difference between the percentages of strains that were resistant to these two agents, i.e., 0.5 and 1.9, respectively. Overall, however, 27.1% of isolates were noted to be intermediate to clarithromycin. Breakpoints for an azithromycin intermediate category have not been defined.

Again, on the basis of the breakpoints defined by NCCLS (21), resistance was more common to TMP-SMX (9.0%) than to chloramphenicol (0.2%), tetracycline (1.3%), or rifampin (0.3%). Eighty-eight (64.2%) of the TMP-SMX-resistant strains produced β -lactamase. With the exception of one TMP-SMX-resistant strain which was found to be resistant to both tetracycline and rifampin, TMP-SMX resistance usually occurred in the absence of resistance to other non- β -lactam agents. Of note, all three chloramphenicol-resistant isolates produced β -lactamase and were also resistant to tetracycline.

A total of 39 β -lactamase-negative strains (4.0% of all β -lactamase-negative isolates) were found to be resistant (MICs, ≥ 4 μ g/ml; $n = 13$) or intermediate (MICs, 2 μ g/ml; $n = 26$) to ampicillin and thus were categorized as BLNAR. Ampicillin MIC determinations were repeated with these 39 strains in a second lot of HTM broth and never varied by more than ± 1 twofold concentration increment (data not shown). Twenty-eight of these 39 strains were resistant to A-C (MICs, ≥ 8 μ g/ml); the A-C MICs for the remaining 11 strains categorized as BLNAR were 4.0 μ g/ml. By comparison, the modal ampicillin and A-C MICs for β -lactamase-negative, ampicillin-susceptible strains (i.e., strains for which ampicillin MICs were ≤ 1.0 μ g/ml) were 0.25 to 0.5 and 1.0 μ g/ml, respectively.

Further evidence that these 39 apparent BLNAR strains were indeed distinct from typical ampicillin-susceptible, β -lactamase-negative organisms could be found in comparisons of the activities of the cephalosporins against these two groups of *H. influenzae*. The modal MICs of cefaclor, loracarbef, cefprozil, cefuroxime, cefixime, and cefpodoxime for BLNAR strains were 16 to 32, 16, 32, 4 to 8, 0.06, and 0.25 to 0.5 μ g/ml, respectively. In all cases, these values were much higher than the modal MICs obtained with the same agents versus ampi-

TABLE 3. MICs of 15 antimicrobial agents for 1,537 recent clinical isolates of *H. influenzae*

Antimicrobial agent	MIC (μ g/ml)							
	β -Lactamase-negative isolates ($n = 977$)				β -Lactamase-positive isolates ($n = 560$)			
	50%	90%	Geometric mean	Range	50%	90%	Geometric mean	Range
Ampicillin	0.25	1	0.38	≤ 0.06 -16	64	≥ 256	62.3	2- ≥ 256
A-C	1	2	1.1	0.06- ≥ 32	1	4	1.5	0.25-16
Cefaclor	4	16	4.6	0.25- ≥ 256	4	32	6.6	0.25-128
Loracarbef	2	16	3.2	0.12- ≥ 128	4	64	5.7	0.25- ≥ 256
Cefprozil	8	16	6.5	0.25-128	8	64	12.0	0.5- ≥ 256
Cefuroxime	1	4	1.4	0.03- ≥ 32	1	4	1.3	0.06- ≥ 32
Cefixime	0.03	0.06	0.04	≤ 0.004 - ≥ 8	0.03	0.06	0.04	≤ 0.004 - ≥ 8
Cefpodoxime	0.12	0.25	0.1	≤ 0.004 - ≥ 4	0.06	0.12	0.09	≤ 0.004 - ≥ 4
Erythromycin	8	8	6.4	0.12- ≥ 64	8	8	6.4	0.25- ≥ 64
Azithromycin	2	2	1.7	0.06- ≥ 64	2	2	1.6	≤ 0.015 - ≥ 32
Clarithromycin	8	16	9.0	≤ 0.06 - ≥ 256	8	16	9.6	0.12- ≥ 128
TMP-SMX	< 0.06	0.25	0.12	≤ 0.06 -32	≤ 0.06	8	0.18	≤ 0.06 -32
Chloramphenicol	0.5	1	0.54	0.12-4	0.5	1	0.56	≤ 0.06 -16
Tetracycline	1	1	0.82	0.12-128	1	1	0.87	0.12-128
Rifampin	0.25	0.25	0.20	≤ 0.03 - ≥ 64	0.25	0.25	0.20	≤ 0.03 -16

cillin-susceptible isolates, i.e., 4, 2 to 4, 4, 1.0, 0.03, and 0.06 to 0.12 $\mu\text{g/ml}$, respectively. If the 39 BLNAR strains were added to the β -lactamase-positive isolates, the overall frequency of ampicillin resistance was 38.9%.

A striking finding of this study was the identification of 17 β -lactamase-positive strains of *H. influenzae* (3.0% of all β -lactamase-positive isolates) that were resistant to A-C (BLPACR). Upon initial testing, A-C MICs for 12 of these strains were 8 $\mu\text{g/ml}$, and A-C MICs were 16 $\mu\text{g/ml}$ for 5 strains. The A-C MICs for these 17 strains were corroborated by repeat microdilution testing in a second lot of HTM broth and in Mueller-Hinton broth containing 3% lysed horse blood plus 15 μg of NAD per ml. In addition, broth microdilution determinations of A-C MICs were accomplished with these strains by using commercially prepared panels containing dehydrated antibiotic (Just-One) reconstituted with a third lot of HTM broth. Finally, A-C MICs were determined independently in a second laboratory, the University of Texas Health Science Center at San Antonio (11a), by the microdilution method in HTM broth. The results of this extensive secondary testing were judged to corroborate the initial MIC determinations (data not shown).

For all 17 BLPACR strains, ampicillin MICs were ≥ 128 $\mu\text{g/ml}$. By contrast, the modal ampicillin MIC for β -lactamase-positive strains that were susceptible to A-C (MICs, ≤ 4 $\mu\text{g/ml}$) was 32 $\mu\text{g/ml}$. The modal MICs of cefaclor, loracarbef, cefprozil, cefuroxime, and cefpodoxime (i.e., 16, 16, 32, 4, and 0.25 $\mu\text{g/ml}$, respectively) were consistently higher for β -lactamase-positive, A-C-resistant strains than the modal MICs of the same agents for β -lactamase-positive, A-C-susceptible isolates (i.e., 4, 4, 8, 1, and 0.06 $\mu\text{g/ml}$, respectively). The 17 BLPACR strains when added to the BLNAR strains that were also resistant to A-C, yielding an overall prevalence of A-C resistance of 4.5%.

DISCUSSION

The first recognition of β -lactamase-mediated ampicillin resistance with *H. influenzae* occurred in 1974 (15, 27). It is now understood that this organism may produce either of two β -lactamases, TEM-1 or ROB-1 (14, 18, 24). One study revealed that ca. 93% of β -lactamase-producing strains produced the TEM-1 enzyme, and the remainder produced the ROB-1 enzyme (26). Eight large systematic national surveillance studies of the antibiotic resistance of *H. influenzae* isolates were conducted in North America between 1982 and 1993; five in the United States (1, 8, 9, 12, 23) and three in Canada (25, 26, 28). When restricted to non-type b strains of *H. influenzae*, increases in β -lactamase-mediated ampicillin resistance of ca. 1 to 3% per year have occurred, reaching a level of 33% in 1993. The results of the current investigation suggest that the prevalence of β -lactamase-mediated ampicillin resistance among non-type b strains continues to grow and now exists at a level of 36.4% overall in the United States. The issue of antibiotic resistance of *H. influenzae* strains is appropriately restricted to non-type b strains, since infections most commonly caused by encapsulated type b isolates (i.e., meningitis, epiglottitis, facial cellulitis, and bacteremia) occur very infrequently today in developed countries such as the United States because of the use of protein-conjugated capsular antigen vaccines (3).

Six cephalosporins were examined in this study. They could be categorized into three groups on the basis of their activities against *H. influenzae*. Loracarbef, cefaclor, and cefprozil had MIC₉₀s of 16 to 64 $\mu\text{g/ml}$, the combined percentages of strains intermediate and resistant to these drugs were 16.3 to 29.8, and

the activities of the drugs appeared to be diminished by β -lactamase production. The MIC₉₀ of cefuroxime was 4 $\mu\text{g/ml}$; 1.5% of strains were resistant and 4.9% were intermediate to cefuroxime, and the MIC₅₀s, MIC₉₀s, and geometric mean MICs were essentially the same with β -lactamase-positive and -negative strains. Cefixime and cefpodoxime were the most active cephalosporins, with MIC₉₀s of 0.06 to 0.25 $\mu\text{g/ml}$, the frequencies of resistance to these drugs was 0.1 to 0.3%, and no β -lactamase effect was detected.

This study revealed differences in the activities of three macrolide agents against *H. influenzae* when they were assessed on the basis of MICs. Azithromycin was consistently fourfold more active than erythromycin, which in turn was slightly more active than clarithromycin. These relationships were clear from comparisons of MIC₅₀s, MIC₉₀s, and geometric mean MICs, strain-by-strain comparisons of MICs, or comparisons of the modal MICs derived from the unimodal MIC distributions obtained with all three of the macrolides versus the collection of isolates examined in this study. On the basis of NCCLS-defined MIC breakpoints (21), 0.5% of strains would have been judged to be resistant to azithromycin and 1.9% would have been judged to be resistant to clarithromycin. No intermediate category has been defined for azithromycin; for clarithromycin, 27.1% of strains overall were classified as intermediate (MICs, 16 $\mu\text{g/ml}$). No interpretive breakpoints have been established for erythromycin (21). The question is whether these differences in *in vitro* macrolide activity translate into differences in efficacy in treating *H. influenzae* infections. This question is germane insofar as the MICs determined with clarithromycin alone, as was the case in this study, may underestimate the activity of this agent *in vivo*, since the additive or synergistic effect of the 14-OH metabolite of clarithromycin, which occurs as a result of hepatic metabolism, is not taken into consideration (10). This question can only be answered by controlled clinical trials that directly compare these agents.

The overall frequencies of resistance to other non- β -lactam agents were as follows: TMP-SMX, 9.0%; chloramphenicol, 0.2%; tetracycline, 1.3%; and rifampin, 0.3%. These levels of resistance are similar to those reported in a large surveillance study conducted in the United States in 1992 and 1993 and suggest that the frequencies of resistance to these agents are not changing (1).

Of particular concern in this study was the recovery of 39 apparent BLNAR strains of *H. influenzae* (4.0% of β -lactamase-negative isolates and 2.5% of all isolates). These were strains for which ampicillin MICs were 2 $\mu\text{g/ml}$ (i.e., intermediate) or ≥ 4 $\mu\text{g/ml}$ (resistant). The actual distribution of MICs for isolates in this group was 2 $\mu\text{g/ml}$ ($n = 25$), 4 $\mu\text{g/ml}$ ($n = 9$), 8 $\mu\text{g/ml}$ ($n = 1$), and 16 $\mu\text{g/ml}$ ($n = 3$). Heretofore, BLNAR strains have not been recovered frequently in North America, with frequencies of $\leq 0.1\%$ reported in several recent national surveillance studies (1, 8, 12, 25, 26). Because of the obvious importance of our observations, determinations of the ampicillin MICs for these strains were repeated with a second lot of HTM broth. The β -lactamase assay was also repeated. The results of this secondary testing corroborated the initial results. It appears that BLNAR strains of non-type b *H. influenzae* are now present in significant numbers in the United States. No geographic or institutional clustering of these strains was recognized.

Examination of an ampicillin MIC frequency distribution plot derived from the data generated with β -lactamase-negative strains in this study revealed a unimodal population with a conspicuous modal MIC of 0.25 to 0.5 $\mu\text{g/ml}$. The MICs for the 39 BLNAR isolates were clustered at the higher end of this

distribution. The question that arises is how different are the BLNAR strains from typical β -lactamase-negative, ampicillin-susceptible isolates? To answer this question, the MICs of six cephalosporins for the BLNAR strains were compared with those obtained for β -lactamase-negative, ampicillin-susceptible strains. In all cases, the MICs obtained for organisms in the first group were significantly higher than those for organisms in the second group; to wit, it appears that the BLNAR strains identified in this study truly do represent a distinct subpopulation of *H. influenzae* isolates. Alterations in penicillin-binding proteins have been proposed as a mechanism of resistance in BLNAR isolates of *H. influenzae* (5, 16, 19, 22). Our observations concerning the phenotypic expression of resistance are consistent with such a mechanism.

A second striking observation in this study was the recovery of 17 strains of non-type b β -lactamase-producing *H. influenzae* which appeared to be resistant to the combination A-C. Extensive secondary testing confirmed our initial findings with these strains. We have coined the acronym BLPACR as a designation for these strains. Two isolates of non-type b *H. influenzae* with a comparable phenotype have previously been recovered from human specimens in San Antonio, Tex. (11a). However, we are not aware of any published descriptions of such strains.

Potential explanations for this phenotype include hyperproduction of a TEM-1 or ROB-1 β -lactamase, production of an altered TEM-1 or ROB-1 β -lactamase that has become resistant to clavulanate inhibition, production of a completely novel β -lactamase that is not effectively inhibited by clavulanate, or elaboration of altered penicillin-binding proteins superimposed on typical TEM-1 or ROB-1 β -lactamase production. Efforts to delineate the precise mechanism of resistance in these strains have been undertaken.

Our observations regarding the emergence of BLNAR and BLPACR strains of *H. influenzae*, if corroborated, have numerous ramifications. With the exception of cefixime and cefpodoxime, the in vitro activities of all of the β -lactams examined in this study, including the β -lactamase inhibitor combination A-C, were significantly diminished against these isolates. This may be of clinical significance. Furthermore, if BLNAR and BLPACR strains of *H. influenzae* become common, laboratory strategies for in vitro susceptibility testing may have to change. Specifically, isolates of *H. influenzae* found to be β -lactamase negative could not be considered a priori to be susceptible to ampicillin, A-C, and the six oral cephalosporins examined in this study. Rather, susceptibility tests which directly assess the activities of these agents would be needed. Similarly, it would be inappropriate to consider β -lactamase-positive strains to be uniformly susceptible to A-C. Again, a direct test of A-C activity would be necessary.

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REFERENCES

- 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.
- Bell, S. M., and D. Plowman. 1980. Mechanisms of ampicillin resistance in *Haemophilus influenzae* from the respiratory tract. *Lancet* **i**:279.
- Black, S. B., H. R. Shinefield, and The Kaiser Permanente Vaccine Study Group. 1992. Immunization with oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine on a large HMO population: extended follow-up and impact on *Haemophilus influenzae* disease epidemiology. *Pediatr. Infect. Dis.* **11**:610-613.
- Campos, J. M. 1995. *Haemophilus*, p. 556-565. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. ASM Press, Washington, D.C.
- Clairoux, N., M. Picard, A. Brochu, N. Rousseau, P. Gourde, D. Beauchamp, T. R. Parr, Jr., M. G. Bergeron, and F. Malouin. 1992. Molecular basis of non- β -lactamase-mediated resistance to β -lactam antibiotics in strains of *Haemophilus influenzae* isolated in Canada. *Antimicrob. Agents Chemother.* **36**:1504-1513.
- Doern, G. V. 1988. Antimicrobial resistance among isolates of *Haemophilus influenzae* and *Branhamella catarrhalis*. *Clin. Microbiol. Newsl.* **10**:185-187.
- Doern, G. V. 1995. Trends in antimicrobial susceptibility of bacterial pathogens of the respiratory tract. *Am. J. Med.* **99**:6B-6S.
- Doern, G. V., J. H. Jorgensen, C. Thornsberry, D. A. Preston, T. Tubert, J. S. Redding, and L. A. Maher. 1988. National collaborative study of the prevalence of antimicrobial resistance among clinical isolates of *Haemophilus influenzae*. *Antimicrob. Agents Chemother.* **32**:180-185.
- Doern, G. V., J. H. Jorgensen, C. Thornsberry, D. A. Preston, and the *Haemophilus influenzae* Surveillance Group. 1986. Prevalence of antimicrobial resistance among clinical isolates of *Haemophilus influenzae*: a collaborative study. *Diagn. Microbiol. Infect. Dis.* **4**:95-107.
- Hardy, D. J., R. N. Swanson, R. A. Rode, K. Marsh, N. L. Shipkowitz and J. J. Clement. 1990. Enhancement of the in vitro and in vivo activities of clarithromycin against *Haemophilus influenzae* by 14-hydroxy-clarithromycin, its major metabolite in humans. *Antimicrob. Agents Chemother.* **34**:1407-1413.
- Jorgensen, J. H. 1992. Update on mechanisms and prevalence of antimicrobial resistance in *Haemophilus influenzae*. *Clin. Infect. Dis.* **14**:1119-1123.
- Jorgensen, J. H. Personal communication.
- 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.
- Jorgensen, J. H., J. S. Redding, L. A. Maher, and A. W. Howell. 1987. Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. *J. Clin. Microbiol.* **25**:2105-2113.
- Juteau, J., and R. C. Levesque. 1990. Sequence analysis and evolutionary perspectives of ROB-1 β -lactamase. *Antimicrob. Agents Chemother.* **34**:1354-1359.
- Khan, W., S. Ross, W. Rodriguez, G. Controni, and A. K. Saz. 1974. *Haemophilus influenzae* type b resistant to ampicillin: a report of two cases. *JAMA* **229**:298-301.
- Malouin, F., A. B. Schryvers, and L. E. Bryan. 1987. Cloning and expression of genes responsible for altered penicillin-binding proteins 3a and 3b in *Haemophilus influenzae*. *Antimicrob. Agents Chemother.* **31**:286-291.
- Markowitz, S. M. 1980. Isolation of an ampicillin-resistant, non- β -lactamase-producing strain of *Haemophilus influenzae*. *Antimicrob. Agents Chemother.* **17**:80-83.
- Medeiros, A. A., R. Levesque, and G. A. Jacoby. 1986. An animal source for the ROB-1 β -lactamase of *Haemophilus influenzae* type b. *Antimicrob. Agents Chemother.* **29**:212-215.
- Mendelman, P. M., D. O. Chaffin, T. L. Stull, C. E. Rubens, K. D. Mack, and A. L. Smith. 1984. Characterization of non- β -lactamase-mediated ampicillin resistance in *Haemophilus influenzae*. *Antimicrob. Agents Chemother.* **26**:235-244.
- Montgomery, K. L., L. Raymundo, Jr., and W. L. Drew. 1979. Chromogenic cephalosporin spot test to detect beta-lactamase in clinically significant bacteria. *J. Clin. Microbiol.* **9**:205-207.

21. **National Committee for Clinical Laboratory Standards.** 1995. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. M7-A4. National Committee for Clinical Laboratory Standards. Villanova, Pa.
22. **Parr, T. R., Jr., and L. E. Bryan.** 1984. Mechanism of resistance of an ampicillin-resistant, β -lactamase-negative clinical isolate of *Haemophilus influenzae* type b to β -lactam antibiotics. *Antimicrob. Agents Chemother.* **25**:747-753.
23. **Rittenhouse, S. F., L. A. Miller, R. L. Kaplan, G. H. Mosely, and J. A. Poupard.** 1995. A survey of β -lactamase-producing *Haemophilus influenzae*. *Diagn. Microbiol. Infect. Dis.* **21**:223-225.
24. **Rubin, L. G., R. H. Yolken, A. A. Medeiros, and E. R. Moxon.** 1981. Ampicillin treatment failure of apparently β -lactamase-negative *Haemophilus influenzae* type b meningitis due to novel β -lactamase. *Lancet* **i**:1008.
25. **Scriver, S. R., D. E. Low, A. E. Simor, B. Toye, A. McGeer, R. Jaeger, and Canadian Haemophilus Study Group.** 1992. Broth microdilution testing of *Haemophilus influenzae* with Haemophilus Test Medium versus lysed horse blood broth. *J. Clin. Microbiol.* **30**:2284-2289.
26. **Scriver, S. R., S. L. Walmsley, C. L. Kau, D. J. Hoban, J. Brunton, A. McGeer, T. C. Moore, E. Witwicki, Canadian Haemophilus Study Group, and D. E. Low.** 1994. Determination of antimicrobial susceptibilities of Canadian isolates of *Haemophilus influenzae* and characterization of their β -lactamases. *Antimicrob. Agents Chemother.* **38**:1678-1680.
27. **Tomeh, M. O. S. E. Starr, J. E. McGowan, Jr., P. M. Terry, and A. J. Nahmias.** 1974. Ampicillin-resistant *Haemophilus influenzae* type b infection. *JAMA* **229**:295-297.
28. **Tremblay, L. D., J. L'Ecuyer, P. Provencher, M. G. Bergeron, and Canadian Study Group.** 1990. Susceptibility of *Haemophilus influenzae* to antimicrobial agents used in Canada. *Can. Med. Assoc. J.* **143**:895-901.