

Serotype and Ampicillin Susceptibility of *Haemophilus influenzae* Causing Systemic Infections in Children: 3 Years of Experience

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Over a 3-year period, 96% of systemic infections in children caused by *Haemophilus influenzae* were of serotype b. Of 346 invasive infections, 15 (4%) were caused by non-type b *H. influenzae*. The monthly prevalence of ampicillin resistance in all isolates was highly variable (0 to 63%). Ampicillin resistance in *H. influenzae* causing invasive disease occurred in 13% of non-type b and 21.8% of type b isolates. There was no significant difference ($\chi^2 = 0.21$; $P > 0.10$) in the rate of ampicillin resistance between type b and non-type b *H. influenzae* causing systemic illness in children over a 3-year period.

Reports of systemic disease caused by *Haemophilus influenzae* in children correctly point out that the majority of such infections are caused by serotype b (5, 11). Weinstein estimated the frequency of type b isolates from children 6 months to 3 years of age to be 95% (16). Turk and May (13) reported that all *H. influenzae* infections in children are caused by encapsulated strains and that most are caused by those strains which elaborate the type b polysaccharide capsule. Conversely, *H. influenzae* types a, c, d, e, and f and nontypeable strains are most commonly associated with localized infections such as otitis media, sinusitis, and conjunctivitis in children and bacteremic illness in adults (10, 14). We have documented carefully the prevalence of *H. influenzae* disease in children at Texas Children's Hospital, Houston, for 3 years. The distribution of serotypes of these isolates causing invasive disease and the prevalence of ampicillin-resistant strains over this time period are reported.

MATERIALS AND METHODS

Bacterial strains. All strains identified as *H. influenzae* isolated by the Clinical Microbiology Laboratory, Department of Pathology, Texas Children's Hospital, between 1 July 1978 and 30 June 1981 were included in the study. Strains were identified as *H. influenzae* by Gram stain, microscopic morphology, requirement for nicotinamide adenine dinucleotide, and lack of ability to synthesize porphobilinogen and porphyrins from δ -aminolevulinic acid (6, 7). The strains were serotyped from overnight broth cultures by countercurrent immunoelectrophoresis with antisera obtained from Hyland Laboratories (type b) and Burroughs Wellcome Co. (types a, c, d, e, and f) (9). Each strain was tested for the elaboration of β -lactamase by the acidometric

method of Escamilla (4). Ampicillin susceptibility was confirmed by the disk susceptibility method of Bauer and Kirby (1). Each strain was biotyped by the method of Kilian, with tests to detect tryptophanase (indole), urease, and ornithine decarboxylase (7).

Patients. Information on the source of the isolate, clinical diagnosis, and date of admission for each patient was obtained from the medical record. Isolates from multiple sites from the same patient were not included as separate infections. Strains were considered to cause invasive disease if they were isolated from cerebrospinal fluid, blood, or aspirates from abscesses, lung, joint, bone, or cellulitis. We excluded *H. influenzae* isolates from the eye and upper respiratory tract from consideration regardless of the clinical diagnosis.

RESULTS

In a 3-year period, 346 *H. influenzae* isolates were obtained from 345 patients with systemic disease. One patient had recurrent *H. influenzae* type b infection. *H. influenzae* type b accounted for 331 (95.7%) of the 346 isolates. Meningitis was the predominant clinical diagnosis (64.5%), followed by pneumonia (9.8%), septicemia (8.7%), and cellulitis (8.7%). Infections caused by non-type b *H. influenzae* included one case of meningitis, five of septicemia, five of pneumonia, one of septic arthritis, and three of abscess.

The biotype, serotype, and ampicillin susceptibility of *H. influenzae* non-type b isolated from sites of serious infection and the age and diagnosis of those patients are shown in Table 1. All strains were nontypeable *H. influenzae*, except for one serotype f strain which caused septicemia in a 4-year-old female with underlying common variable immune deficiency. Two of the children with septicemia were neonates. There

TABLE 1. Source of isolate, patient age, ampicillin susceptibility, serotype, and biotype of *H. influenzae* non-type b strains causing systemic infections in children

Patient no.	Source of isolate	Age	Sex	β -Lactamase production ^a	Serotype ^b	Biotype	History or diagnosis
1	Blood	2 yr	F	-	NT	II	Sternal wound
2	Blood	1 day	M	-	NT	III	Neonate
3	Blood	4 yr	F	-	f	V	Immunoglobulin A and G immune deficiency
4	Blood	1 day	F	-	NT	IV	Neonate (premature)
5	Blood	11 yr	M	-	NT	IV	Acute lymphocytic leukemia
6	Cerebrospinal fluid	21 mo	F	-	NT	II	Meningitis
7	Blood	2 yr	F	-	NT	I	Pneumonia
8	Blood	6 mo	F	+	NT	III	Pneumonia
9	Pleural fluid	2 yr	M	-	NT	II	Pneumonia
10	Pleural fluid	19 yr	M	-	NT	I	Chronic pulmonary disease
11	Pleural fluid	9 yr	M	-	NT	V	Lung cyst
12	Abscess	6 yr	F	+	NT	I	Thyroglossal duct cyst
13	Abscess	11 mo	M	-	NT	I	Rectal abscess
14	Abscess	18 mo	M	-	NT	I	Rectal abscess
15	Joint fluid	3 yr	F	-	NT	III	Septic arthritis

^a +, Positive; -, negative.

^b NT, Nontypeable.

were no differences in the number of males versus females with systemic infections caused by non-type b *H. influenzae* (seven males, eight females). In addition to these invasive isolates, *H. influenzae* was isolated from 41 middle ear aspirates obtained by myringotomy or tympanocentesis: 5 type b, 1 type f, 1 type a, and 34 nontypeable strains.

Ampicillin resistance mediated by mechanisms other than β -lactamase production was not encountered in this 3-year series of *H. influenzae* strains isolated from children with

invasive disease. Altogether, 2 of 15 (13.3%) non-type b and 72 of 331 (21.8%) type b *H. influenzae* isolates causing serious infections in children were resistant to ampicillin. There was no statistically significant difference in the rate of ampicillin resistance between type b and non-type b *H. influenzae* isolates ($\chi^2 = 0.21$; $P > 0.10$). The monthly prevalence of ampicillin resistance in *H. influenzae* isolates was highly variable (Fig. 1). During 8 separate months, no ampicillin resistance was detected, and during 7 other months, the rate of resistance to ampicillin

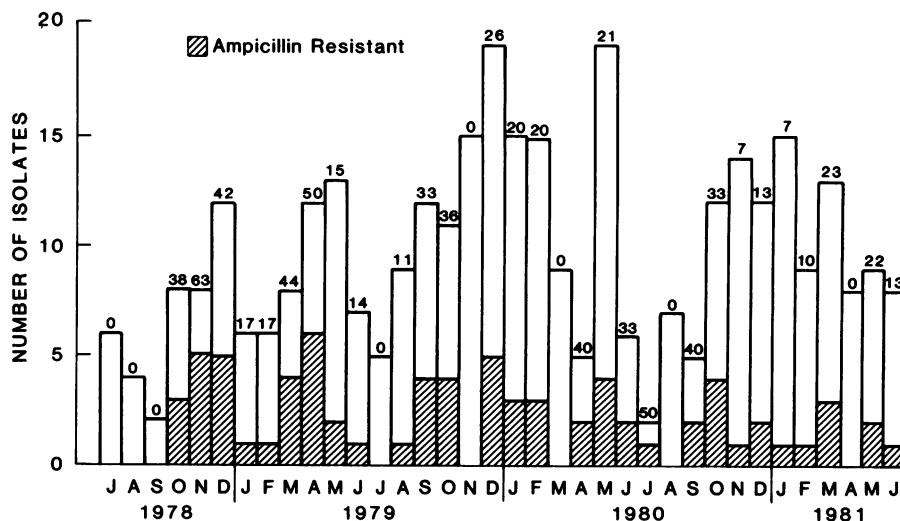


FIG. 1. Monthly isolation and prevalence of ampicillin resistance of *H. influenzae* causing systemic illness in children at Texas Children's Hospital. Isolates include serotype b, non-serotype b, and nontypeable strains. Numbers are the percentages of ampicillin-resistant strains.

was $\geq 40\%$. The overall rate of ampicillin resistance during this 3-year period was 21.4%.

DISCUSSION

H. influenzae serotype b is the major cause of systemic disease in children (11, 13). Precise figures on serious invasive disease in children caused by *H. influenzae* non-type b are not available generally. Lerman et al. (8) found that in Omaha, 91% of systemic *H. influenzae* infections were caused by type b organisms, 3% were caused by serotype a, c, or d, and 6% were caused by nontypeable isolates. This 5-year retrospective review was based on information obtained by the bacteriological and serological methodologies of three different hospitals and thus may reflect the inaccuracies of the various techniques. Further, these studies included isolates from both adults and children and made the assessment of disease caused by non-type b *H. influenzae* in children difficult because adults more often have infections caused by nontypeable *H. influenzae* (16). This report, based on 3 years of careful observation in our institution that showed that 96% of *H. influenzae* systemic diseases in children were caused by type b strains, is in very close agreement with the 95% reported by Weinstein in 1970 (16). Only 1 of the 15 non-serotype b isolates causing systemic disease could be typed (type f). Of the 15 patients with non-serotype b infections, either 6 were neonates or had some underlying chronic disease. There was no predominant biotype among these non-type b *H. influenzae* isolates.

Ampicillin resistance is an important consideration in the treatment of infections caused by *H. influenzae*, and resistant isolates from children have been reported in systemic infections caused by type b organisms as well as by nontypeable isolates from such sites as the middle ear (12). During 1975 and 1976, a nationwide survey revealed that the overall prevalence of ampicillin resistance was 4.5%, with a significantly higher rate (9%) in the region encompassing Texas (15). A more recent survey (1978) found a resistance rate of 18% nationwide (3). Both of these reports speculate that these may be inflated estimates of ampicillin resistance. The report by Ward et al. (15) found a much higher reported rate of ampicillin resistance from hospitals in the western survey region which relied solely on disk diffusion susceptibility tests than from those also employing β -lactamase tests (12 versus 3.7%). A recent report from the Centers for Disease Control (3) proposes that the high rate of reported ampicillin resistance may be due to preferential reporting of the ampicillin-resistant strains which cause meningitis.

Over a 3-year period, we have monitored very

carefully ampicillin resistance among *H. influenzae* isolates by both disk diffusion tests and β -lactamase tests. In our study, we found no differences between the results of disk diffusion or β -lactamase antibiotic susceptibility tests. On a monthly basis, ampicillin resistance among the isolates, which caused systemic illness in children was highly variable. Although we found that all ampicillin resistance was mediated by β -lactamase, non- β -lactamase-mediated ampicillin resistance in *H. influenzae* isolates has been reported, but it is rare in this country and generally has not been associated with systemic infection (2). Further, the findings that 16.1% of nontypeable strains from middle ear aspirates and that 2 (13.3%) of 15 non-type b isolates from sites of invasive disease were resistant to ampicillin indicate that individual antibiotic susceptibility testing of all isolates, regardless of serotype, is necessary. Ampicillin resistance in *H. influenzae* varies between regions and institutions. This point is emphasized in a recent report by Wallace et al., who found that only 2% of 45 isolates of *H. influenzae* (type b and non-type b) in adults from the same community (Houston, Tex.) were resistant to ampicillin (14).

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