Ampicillin-Resistant *Haemophilus paraphrophilus* Laryngo-Epiglottitis

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A case of life-threatening laryngo-epiglottitis is reported, caused by ampicillin-resistant *Haemophilus paraphrophilus*. Clinicians and microbiologists should be aware of a beta-lactamase-mediated resistance among *Haemophilus* species other than *H. influenzae*.

The emergence of ampicillin-resistant *Haemophilus* organisms was first recognized in late 1973. The reports have been limited to *Haemophilus influenzae* usually isolated from normally sterile body sites, particularly the cerebral spinal fluid (1, 3, 5, 8). The incidence of ampicillin-resistant *H. influenzae* has escalated to nearly 10% in some United States populations, and resistant strains have been reported from 23 states and the District of Columbia (1). Since *H. influenzae* serotype b and rarely other serotypes cause the vast majority of clinical *Haemophilus* infections, little data exist about ampicillin resistance among other *Haemophilus* species.

We report the first case to our knowledge of laryngo-epiglottitis caused by a beta-lactamase-producing *Haemophilus paraphrophilus*.

REPORT OF A CASE

A 6-month-old male was admitted to Kaiser Foundation Hospital in Portland, Ore., on 17 October 1975. Physical examination revealed an infant male with Down's syndrome. Respirations were markedly labored, with stridor and moderate peripheral cyanosis. Several retractions were later sited, and the patient was placed on 30% O₂ in a mist tent. Temperature was 101°F (38.3°C), and white blood count was 15,700 with a left shift (including 16% bands). Throat culture was taken and the patient was given 1 g of ampicillin per day.

Over the next 12 h, stridor worsened and the baby was intubated. An edematous, slightly hyperemic epiglottis and subglottis region was visualized. The antibiotic and endotracheal intubation were continued for 3 days, at which time the tube was removed. By 24 October 1975, the patient's condition worsened, thus requiring reintubation; the temperature remained 99 to 101°F (37.2 to 38.3°C). Direct visualization of the epiglottic area showed no resolution of edema or hyperemia. Gentamicin was added to the ampicillin dosage on that date. After 2 days, a tracheostomy was performed to minimize the danger of subglottic tube-induced stenosis.

Cultures (five taken from the epiglottic area at

the second intubation) grew predominantly *H. paraphrophilus*, beta-lactamase positive. The patient's temperature returned to normal on 27 October 1975 after 3 days of combined ampicillin and gentamicin therapy. Follow-up laryngoscopy on 5 and 11 November failed to demonstrate edema or erythema. Laryngeal cultures were negative for beta-lactamase-producing *H. paraphrophilus*. Tracheostomy was discontinued on the 24th hospital day and the boy returned home on 13 November 1975.

MATERIALS AND METHODS

Organism identification. The reported beta-lactamase-positive *H. paraphrophilus* isolate and 29 additional *Haemophilus* strains were initially isolated on chocolate 5% sheep blood agar containing 1.0% IsoVitalX (BBL). Growth factor requirements were determined on brain heart infusion agar using X-, V-, and XV-impregnated paper disks (Difco). Rabbit blood agar (5%) was used for the hemolysis testing. A CO₂-dependence test was performed according to the method of Zinneman et al. (10). Nutrient agar plates were prepared with and without 0.6% NaCl. Test organisms and controls were streaked onto the agar surface, followed by the placement of a V-factor disk. Salt-containing and -deficient media were incubated in 10% CO₂ and ambient air (35°C) for 72 h, and then the growth was determined. Speciation was made by the following criteria: *H. parainfluenzae*, growth on all media; *H. paraphrophilus*, growth on all media except salt-deficient agar incubated in ambient air.

Additional biochemical tests were performed on the reported isolate, eight other beta-lactamase-negative *H. paraphrophilus* strains, and 21 *H. parainfluenzae* isolates. Acid production assays from eight carbohydrates were done in microdilution (200-μl volume) using Canalco-Ames trays. The basal broth was peptone-yeast containing 5% Fildes reagent and 1% tested carbohydrate. Inoculated trays were incubated in ambient air for 5 days, and the medium pH was determined by pH meter. A pH drop of greater than 1 pH unit was considered an acid reaction, and a pH drop of 0.5 to 1 pH units was a weak acid shift. Standard methods were used for catalase, indole, nitrate, and urea.

Beta-lactamase tests. *Haemophilus* isolates were

tested for beta-lactamase by two methods. The routinely used method was that described by Thornsberry and Kirven (6). A second confirming test was then applied on all acidometric-positive isolates. This chromogenic cephalosporin (Glaxo compound 87/312) technique was described by O'Callaghan et al. in 1972 (4).

Antibiotic susceptibility testing. Ampicillin, chloramphenicol, gentamicin, and tetracycline minimum inhibitory concentrations were determined in Mueller-Hinton broth supplemented with 5% Fildes reagent. The microdilution technique was employed using an inoculum size of 5×10^4 organisms/ml, incubated for 24 h in ambient air (7).

RESULTS AND DISCUSSION

The discovery of this unusual infection raises several provocative clinic- and laboratory-oriented issues. First, a *Haemophilus* species other than *H. influenzae* may cause infectious

airway obstruction. Several previous reports have documented the potential pathogenicity of such *Haemophilus* species (2, 9). However, we have been unable to find a case reporting laryngo-epiglottitis caused by *H. paraphrophilus*.

Secondly, and similarly unique, was the finding of a beta-lactamase-mediated ampicillin resistance in this organism. The resistance was confirmed by broth dilution susceptibility tests. The minimum inhibitory concentration results of four antibiotics were as follows: ampicillin, $>128 \mu\text{g/ml}$; chloramphenicol, $0.5 \mu\text{g/ml}$; gentamicin, $0.5 \mu\text{g/ml}$; tetracycline, $0.5 \mu\text{g/ml}$. The use of these beta-lactamase tests and confirming antibiotic susceptibility methods have been limited in most laboratories to *H. influenzae* isolates and/or *Haemophilus*-like organisms found in cerebral spinal fluid or blood cultures. With the discovery of ampicillin-resistant, pathogenic *H. paraphrophilus*, these tests must be applied to the screening of selected respiratory tract, wound and deep-tissue isolates. We recommend the routine beta-lactamase testing of any respiratory tract *Haemophilus* species when isolated as the predominant organism or when persisting while the patient is on ampicillin therapy. In addition, these tests should be performed on all *Haemophilus* species from normally sterile body sites, wounds, and deep-tissue infections.

Table 1 lists the identifying characteristics of *H. paraphrophilus* and other V-factor-dependent *Haemophilus* species. *H. paraphrophilus* would be erroneously speciated by nearly all clinical laboratories. Without the cumbersome, time-consuming CO_2 -dependence test, all such isolates would be reported as *H. parainfluenzae*. Our organism was referred to the Center

TABLE 1. Identifying characteristics of V-factor-dependent *Haemophilus* species

Species	Tests			
	Hemolysis (rabbit blood)	CO_2 dependence on salt-deficient media	Catalase	Serum dependence
<i>Haemophilus parainfluenzae</i>	—	—	+	—
<i>H. paraphrophilus</i>	—	+	+	—
<i>H. parahaemolyticus</i>	+	—	+	—
<i>H. paraphrophae-molyticus</i>	+	+	+	—
<i>H. parasuis</i>	—	—	+	+
<i>H. paragallinarum</i>	—	+	—	+

TABLE 2. Biochemical and physiological characteristics of *Haemophilus parainfluenzae* and *Haemophilus paraphrophilus* strains including a beta-lactamase-producing isolate

Biochemical test	<i>H. paraphrophilus</i> (reported strain)			<i>H. paraphrophilus</i> (8 strains)			<i>H. parainfluenzae</i> (21 strains)		
	+	—	% +	+	—	% +	+	—	% +
Acid production from:									
Dextrose	1	0	100	8	0	100	21	0	100
Fructose	1	0	100	8	0	100	19	2	90
Lactose	0	1	0	2	6	25	6	15	29
Maltose	1	0	100	8	0	100	19	2	90
Mannitol	0	1	0	1	7	13	0	21	0
Raffinose	0	1	0	4	4	50	16	5	76
Sucrose	1	0	100	8	0	100	21	0	100
Xylose	0	1	0	0	8	0	1	20	5
Catalase	1	0	100	8	0	100	21	0	100
Indole	0	1	0	0	8	0	0	21	0
Nitrate	1	0	100	8	0	100	21	0	100
Urea	0	1	0	4	4	50	11	10	52

^a Includes strong and weak acid production.

for Disease Control, where the correct identification was made and the antibiotic susceptibility results were confirmed. R. Weaver reports (personal communication, 1976) that 49 of 59 *H. parainfluenzae* strains sent to the Center for Disease Control were actually *H. paraphrophilus* when recently reexamined using the CO₂-dependence criteria of Zinneman et al. (10). In clinical respiratory tract cultures, we find 65 to 75% *H. paraphrophilus* among the non-hemolytic, V-factor-dependent *Haemophilus* species isolated. Additional biochemical characteristics are listed in Table 2. No significant differences exist between the *H. paraphrophilus* and *H. parainfluenzae* isolates using these 12 commonly used criteria.

We believe that clinicians should be aware that a *Haemophilus* species other than *H. influenzae* can produce serious clinical infections. Furthermore, the laboratorian must acquire methods of detecting beta-lactamase-mediated ampicillin resistance and be willing to apply the technique to all significant *Haemophilus* isolates.

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