Ampicillin-Resistant \textit{Haemophilus paraphrophilus} Laryngo-
Epiglottitis

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

The emergence of ampicillin-resistant \textit{Haemophilus} organisms was first recognized in late
1973. The reports have been limited to \textit{Haemophilus influenzae} usually isolated from nor-

mally sterile body sites, particularly the cere-

bral spinal fluid (1, 3, 5, 8). The incidence of ampicillin-resistant \textit{H. influenzae} has esca-

dated to nearly 10% in some United States popu-
lations, and resistant strains have been re-

ported from 23 states and the District of Colum-
bia (1). Since \textit{H. influenzae} serotype b and

rarely other serotypes cause the vast majority of clinical \textit{Haemophilus} infections, little data exist about ampicillin resistance among other \textit{Haemophilus} species.

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

REPORT OF A CASE

A 6-month-old male was admitted to Kaiser Foun-
dation 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 cy-

anosis. Several retractions were later noted, and the

patient was placed on 30% \textit{O}$_2$ in a mist tent. Tem-

perature 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 con-

tinued for 3 days, at which time the tube was removed.

By 24 October 1975, the patient’s condition
worsened, thus requiring reintubation; the tempera-

ture remained 99 to 101°F (37.2 to 38.3°C). Direct

visualization of the epiglottic area showed no resol-

ution 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 \textit{H. parar-

phrophilus}, 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 No-

vember failed to demonstrate edema or erythema.

Laryngeal cultures were negative for beta-lacta-

mase-producing \textit{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-lacta-

mase-positive \textit{H. paraphrophilus} isolate and 29 ad-

ditional \textit{Haemophilus} strains were initially isolated

on chocalated 5% sheep blood agar containing 1.0% \textit{IsoVitaleX (BBL). Growth factor requiremen}-
t were determined on brain heart infusion agar using \textit{X,-}, \textit{V,-}, and \textit{XV-impregnated paper disks (Difco). Rabbit
blood agar (5%) was used for the hemolysis testing.

A CO$_2$-dependence test was performed according to

the method of Zinnesman et al. (10). Nutrient agar
plates were prepared with and without 0.6% NaCl.

Test organisms and controls were streaked onto the
gar surface, followed by the placement of a V-factor
disk. Salt-containing and -deficient media were in-
cubated in 10% CO$_2$ and ambient air (35°C) for 72 h,

and then the growth was determined. Speciation

was made by the following criteria: \textit{H. parainflu-

enzae}, growth on all media; \textit{H. paraphrophilus},
growth on all media except salt-deficient agar incu-
bated in ambient air.

Additional biochemical tests were performed on

the reported isolate, eight other beta-lactamase-
negative \textit{H. paraphrophilus} strains, and 21 \textit{H. para-

influenzae} 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% Filde-

s 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. \textit{Haemophilus} isolates were
tested for beta-lactamase by two methods. The routinely used method was that described by Thornberry and Kirven (6). A second confirming test was then applied on all acidoicitive-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 x 10⁴ 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

### Table 1. Identifying characteristics of V-factor-dependent Haemophilus species

<table>
<thead>
<tr>
<th>Tests</th>
<th>Species</th>
<th>Hemolysis (rabit blood)</th>
<th>CO₂ dependence on salt-deficient media</th>
<th>Catalase</th>
<th>Serum dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haemophilus parainfluenzae</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>H. paraphrophilus</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>H. paraparahaemolyticus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>H. paraphrophilus molyticus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>H. parasuis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>H. paragallinarum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>H. paraphrophilus (reported strain)</th>
<th>H. paraphrophilus (8 strains)</th>
<th>H. parainfluenzae (21 strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+*</td>
<td>-</td>
<td>% +</td>
</tr>
<tr>
<td>Acid production from</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Fructose</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Lactose</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Maltose</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose</td>
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<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Xylose</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Catalase</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Indole</td>
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</tr>
<tr>
<td>Nitrate</td>
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<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Urea</td>
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<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Includes strong and weak acid production.

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 µg/ml; chloramphenicol, 0.5 µg/ml; gentamicin, 0.5 µg/ml; tetracycline, 0.5 µg/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 CO₂-dependence test, all such isolates would be reported as H. parainfluenzae. Our organism was referred to the Center
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.

LITERATURE CITED