

*Haemophilus influenzae* TYPE B RESISTANT TO AMPICILLIN  
AND CHLORAMPHENICOL IN AN ORPHANAGE IN THAILAND

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**OBJECTIVE :** Prevent the colonization and dissemination of ampicillin and chloramphenicol resistant *Haemophilus influenzae* type B in an orphanage in Thailand. Characterize the plasmids encoding for  $\beta$  lactamase in *H. flu* and compare these with plasmids encoding for  $\beta$  lactamase plasmids in *Neisseria gonorrhoeae*.

**BACKGROUND :** Epidemics of *Haemophilus influenzae* type B infections have been reported in day-care centers and chronic care facilities in the United States (1-3). Rifampin and sulfamethoxazole-trimethoprim have been used in attempts to eradicate nasopharyngeal carriage of this pathogen in young children (4, 5). Three children died of meningitis caused by *H. flu* type B resistant to ampicillin and chloramphenicol over a four month period in an orphanage in Bangkok, Thailand. Attempts to eradicate this multiresistant pathogen with rifampin, or rifampin and minocycline are described. The sizes of plasmids encoding for ampicillin and chloramphenicol resistance were determined, and compared with plasmids encoding for  $\beta$  lactamase in *Neisseria gonorrhoeae*.

**METHODS :**

Study Population : Between July and October, 1979 three children from Phyathai orphanage in Bangkok were admitted to Phra Mongkutklao hospital with meningitis. *H. flu* type B recovered from the spinal fluid of these three children were resistant to ampicillin and chloramphenicol. All three of these children died. A fourth child from the same orphanage with *H. flu* type B meningitis and septic arthritis, diagnosed by countercurrent immunoelectrophoresis, was admitted to the hospital on November 10. This child's *H. flu* type B infection was successfully treated with rifampin and sulfamethoxazole-trimethoprim.

On November 1, nasopharyngeal (Np) cultures were collected from children at the orphanage. On December 24, children were cultured again, weighed, and treated with either rifampin 10 mg/kg/12 hours, or rifampin 10 mg/kg/12 hours and minocycline 2 mg/kg/12 hours for three days. One week after termination of treatment, Np cultures were obtained. Nurses and aides were cultured on November 1, December 24, and January, and encouraged to bring their children under five years of age to the bacteriology laboratory for culture.

Bacteriology : Np swabs were cultured on chocolate agar, and incubated immediately in 5% CO<sub>2</sub> at 37°C for 24 hours. *H. flu* were identified by gram stain, and nutritional requirements for X and V (6). *H. flu* isolates were serotyped by slide agglutination using commercial *H. flu* typing sera (Difco,

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Detroit, MI). Organisms were tested for  $\beta$  lactamase production by testing broth cultures with nitrocefin solution as previously described (7). Resistance to ampicillin Ap (30  $\mu$ g), and chloramphenicol Cm (30  $\mu$ g) were initially determined by the Kirby Bauer method (8). Resistance to Ap, Cm, Rf, and minocycline Mc were subsequently determined by the plate dilution method employing  $10^5$  and  $10^6$  organisms (9).

Analysis of Plasmids : Sensitive and resistant *H. flu* type B,  $\beta$  lactamase producing *N. gonorrhoeae* isolated in Thailand and the Philippines in 1979, and *E. coli* K12 711 containing plasmids of known molecular weight (R1 drd, 62, RP4, 34, RSF 1030, 5.5, RSF 1010, 4.4, and PMB8, 1.8 and  $3.74 \times 10^6$  daltons) were grown in 50 ml BHI supplemented with 10% lysed sheep blood at 37°C overnight. Bacteria were centrifuged at 5000G for ten minutes. Plasmid DNA (5-20  $\mu$ l) from cleared lysates was subjected to electrophoresis in 0.7% agarose (Seakem, Marine Colloids Inc.), stained with ethidium bromide in water (0.5  $\mu$ g/ml), and photographed under long wave ultraviolet light.

## RESULTS :

Bacteriology : The results of the Np cultures are given in Table 1. Ninety-three children were treated with Rf, and 92 with Rf and Mc. Of eighteen children carrying *H. flu* type B on December 24, eighty-three percent (10/12) of children treated with Rf, and 67 percent (4/6) treated with Rf and Mc had negative Np cultures for *H. flu* type B on January 4. Two children initially colonized with Ap<sub>S</sub> Cm<sub>S</sub> *H. flu* type B were infected with Ap<sub>R</sub> Cm<sub>R</sub> isolates following treatment with either Rf, or Rf and Mc. A third child colonized with an Ap<sub>S</sub> Cm<sub>S</sub> strain was infected with an Ap<sub>R</sub> Cm<sub>R</sub> isolate following treatment with Rf. Fourteen children, who were not originally infected, acquired *H. flu* type B after therapy (one child was not cultured prior to treatment). None of 75 nursing personnel were carriers of *H. flu* type B, however 22 percent (5/23) of their children under five years of age were colonized. Two of these five children were colonized with Ap<sub>R</sub> Cm<sub>R</sub> organisms.

All Ap<sub>R</sub> *H. flu* type B produced  $\beta$  lactamase. Cm<sub>R</sub> isolates were resistant to greater than 3.2  $\mu$ g/ml while sensitive strains were inhibited by 0.4  $\mu$ g/ml. The antibiotic sensitivities of Ap<sub>R</sub> Cm<sub>R</sub>, Ap<sub>R</sub> Cm<sub>S</sub>, Ap<sub>S</sub> Cm<sub>R</sub>, and Ap<sub>S</sub> Cm<sub>S</sub> isolates to Mc and Rf are shown in Table 2. Ninety-seven percent (34/35) of bacteria that were resistant to Ap, Cm, or both had one-fold higher minimum inhibitory concentrations (MIC's) to Mc than eighty-one percent (13/16) of the sensitive strains. Six of 35 resistant isolates were similarly more resistant to Rf than all 16 sensitive strains. There was no consistent difference in MIC's to Rf and Mc between *H. flu* type B isolated from children who were or were not cured, or strains, recovered before or after treatment.

Plasmid : As shown in figure 1, a 4.5 megadalton plasmid was present in *H. flu* type B that produced  $\beta$  lactamase. In addition 2.2 and 1.2 megadalton plasmids were found in isolates that were resistant to chloramphenicol. A 4.5 megadalton plasmid was also present in *N. gonorrhoeae* that produced  $\beta$  lactamase, figure 2.

This study is complete.

Table 1. Results of Nasopharyngeal Cultures at Phyathai Orphanage

<u>Date of culture</u>	<u>11/1/79</u>	<u>12/26/79</u>	<u>1/4/80</u>
		(Pre Rx)	(Post Rx)
Number of children cultured	219	208	198
Children infected with <i>H. flu</i> type B	38 (17%)	18 (9%)	19 (10%)
<i>H. flu</i> B sensitivity			
Ap <sub>r</sub>	0 (0%)	0 (0%)	1 (5%)
Cm <sub>r</sub>	1 (3%)	1 (6%)	3 (16%)
Ap <sub>r</sub> + Cm <sub>r</sub>	18 (47%)	6 (33%)	8 (44%)

Table 2. The Minimum Inhibitory Concentration of 16 Ap<sub>S</sub>Cm<sub>S</sub>,  
 28 Ap<sub>R</sub>Cm<sub>R</sub>, five Ap<sub>S</sub>Cm<sub>R</sub>, and two Ap<sub>R</sub>Cm<sub>S</sub> *H. flu* type B  
 to Minocycline and Rifampin

	Ap <sub>R</sub> Cm <sub>R</sub> (28 <sup>+</sup> )	Ap <sub>R</sub> Cm <sub>S</sub> (2)	Ap <sub>S</sub> Cm <sub>R</sub> (5)	Ap <sub>S</sub> Cm <sub>S</sub> (16)
<u>Minocycline</u>				
1.6*	0	1	0	0
0.2	27 <sup>+</sup>	1	5	3
0.1	1	0	0	13
<u>Rifampin</u>				
0.4	5	1	0	0
0.2	23	1	5	16 *

+ Number of isolates

\* MIC in µg/ml testing with an inoculum of 10<sup>3</sup> bacteria.

MIC's determined with 10<sup>6</sup> bacteria had onefold higher MIC's.

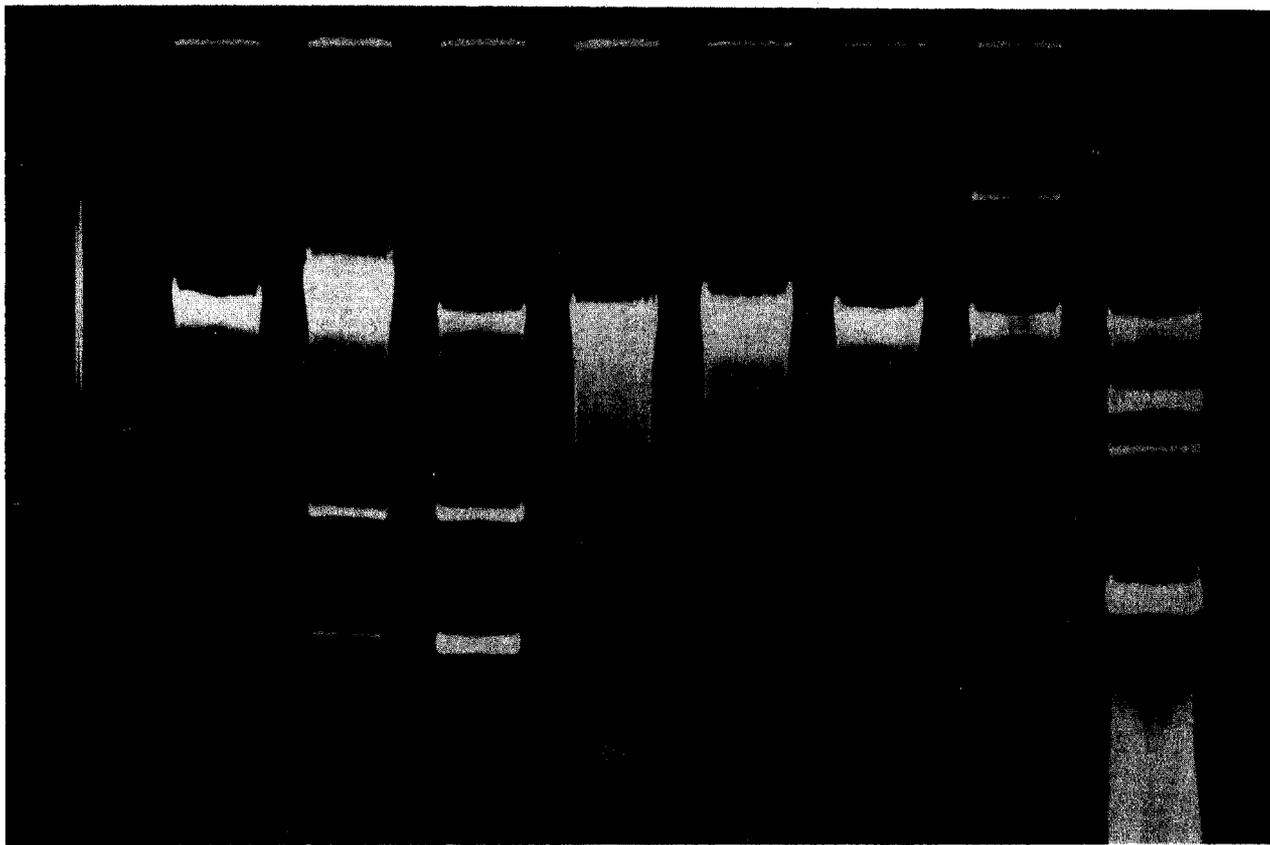


Figure 1. Agarose gel electrophoresis of cleared lysates of sensitive and Ap and/or Cm resistant *H. flu* type B, and *E. coli* K12 711 containing plasmids of known molecular weight. (A) Chromosomal fragments of Ap<sub>s</sub>Cm<sub>s</sub> *H. flu* type B, no plasmids present, (B) Ap<sub>r</sub>Cm<sub>r</sub> *H. flu* type B containing three plasmids (molecular weight, 4.5, 2.2, and 1.2 x 10<sup>6</sup> daltons), (C) Ap<sub>s</sub>Cm<sub>r</sub> *H. flu* type B containing two plasmids (molecular weight 2.2 and 1.2 x 10<sup>6</sup> daltons), (D) *E. coli* 711 containing RSF 1030 (molecular weight 5.5 x 10<sup>6</sup> daltons), (E) *E. coli* 711 containing RSF 1010 (molecular weight 4.4 x 10<sup>6</sup> daltons), (F) *E. coli* 711 containing R1 (molecular weight 62 x 10<sup>6</sup> daltons), (G) *E. coli* 711 containing RP4 (molecular weight 34 x 10<sup>6</sup> daltons) and (H) Lysates of two *E. coli* 711 containing RSF 1010, and PMB 8 (molecular weight 4.4, 3.7 (dimer of PMB 8), and 1.8 x 10<sup>6</sup> daltons).

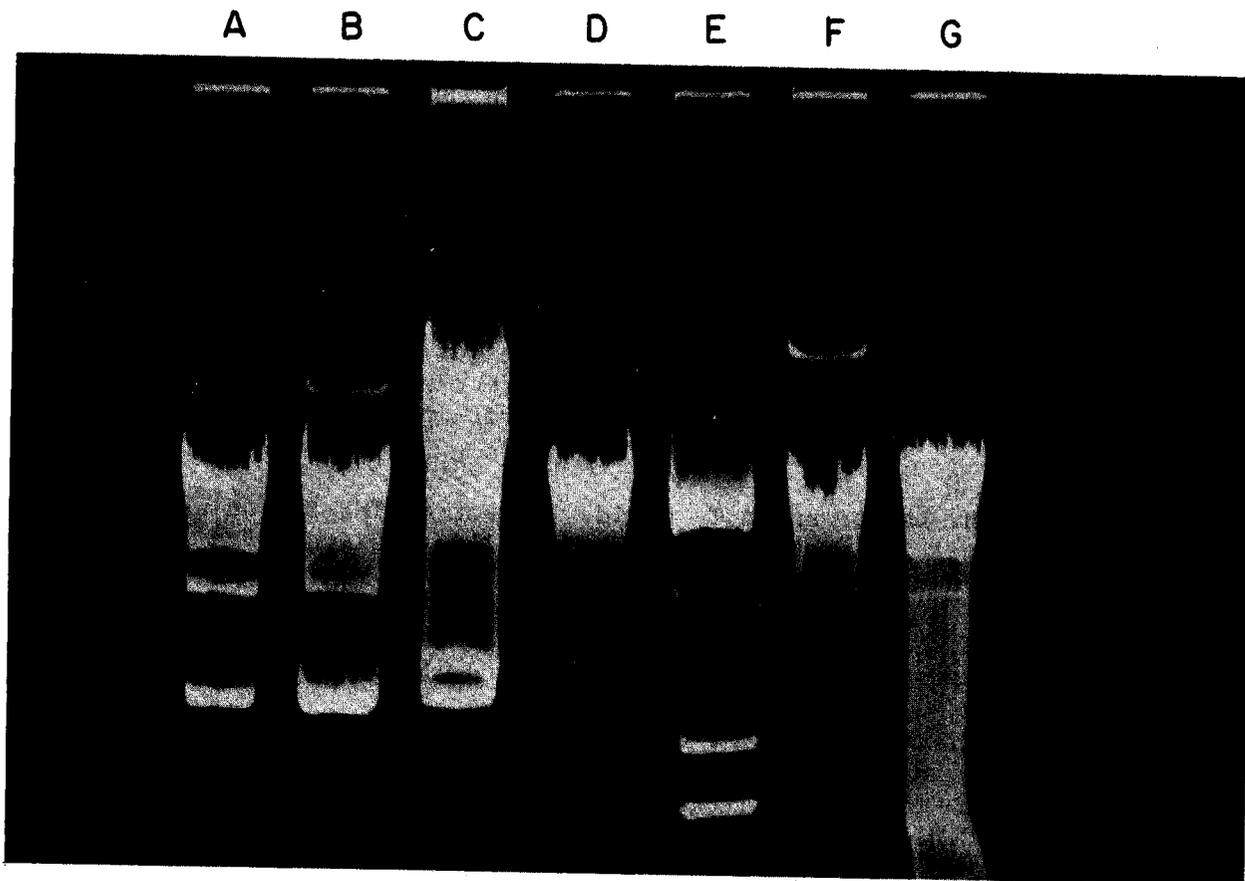


Figure 2. Agarose gel electrophoresis of cleared lysates of  $\beta$  lactamase producing *N. gonorrhoeae*, an  $Ap_{r}Cm_{r}$  *H. flu* type B, and *E. coli* K12 711 containing plasmids of known molecular weight. (A)  $\beta$  lactamase producing *N. gonorrhoeae* isolated in the Philippines containing four plasmids (molecular weight 29, 4.5, and  $2 \times 10^6$  daltons), (B)  $\beta$  lactamase producing *N. gonorrhoeae* isolated in the Philippines containing three plasmids (molecular weight 29, 4.5, and  $2 \times 10^6$  daltons), (C)  $\beta$  lactamase producing *N. gonorrhoeae* isolated in Thailand containing three plasmids (molecular weight 4.5, 2.5, and  $2 \times 10^6$  daltons) (larger plasmids may be hidden by chromosomal DNA), (D)  $\beta$  lactamase producing *N. gonorrhoeae* isolated in Thailand containing a single plasmid (molecular weight  $4.5 \times 10^6$  daltons), (E)  $Ap_{r}Cm_{r}$  *H. flu* type B containing three plasmids molecular weight 4.5, 2.2, and  $1.2 \times 10^6$  daltons), (F) *E. coli* 711 containing R1 (molecular weight  $62 \times 10^6$  daltons), (G) *E. coli* 711 containing RSF 1010 (molecular weight  $4.4 \times 10^6$  daltons).

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