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# Beta-lactamase Producing Haemophilus Influenzae and their Susceptibility to Amoxicillin and Related Antibiotics

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**ABSTRACT.** Seven strains out of 73 strains of H. influenzae isolated from 72 patients produced beta-lactamase and all of the beta-lactamase producing strains were isolated from patients with respiratory diseases. Of 6 strains serotyped by slide agglutination, 2 were type d, the remaining 4 were nontypable.

All of the beta-lactamase producing H. influenzae were resistant to amoxicillin but sensitive to BRL-25,000, Ceftizoxime and Cefmenoxime.

Since ampicillin-resistant strains of H. influenzae type b were first isolated in 1974[1,2], resistant strains have been wide spread and show increasing tendency in recent years[3].

Although resistance is known to result from a beta-lactamase specified by plasmids, the factors leading to the carriage of resistant strains are poorly defined.

We undertook this study to define the prevalence of beta-lactamase producing strains and of resistance to amoxicillin and related antibiotics among H. influenzae obtained from adult patients.

## MATERIALS AND METHODS

Seventy-three isolates of H. influenzae were obtained from 72 different patients at Kawasaki Medical School Hospital between April and December 1981. Most of the isolated H. influenzae patients were from the division of pulmonology. Seventy-two out of 73 isolates were cultured from sputum, and one cultured from puss. Chocolate agar was used as a primary isolation medium for all of the specimens.

H. influenzae isolates were identified on the basis of colony morphology and dependence for growth upon heme and DPM, using a standard strip technique.

H. influenzae isolates were tested for beta-lactamase by a modified 1 min iodometric paper strip test[4,5] and serotyped by the slide agglutination method.

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Sensitivities of H. influenzae isolates to amoxicillin (AMPC), BRL-25,000 (AMPC/clavulanic acid 2 : 1), cefazolin (CEZ), cefotiam (CTM), ceftizoxime (CZX) and cefmenoxime (CMX) were tested by the agar plate dilution method.

## RESULTS

The results of testing 73 isolates are presented in Table 1.

Thirty-eight out of 73 strains were isolated from patients of the division of pulmonology, and all of 7 beta-lactamase producing strains (9.6%) were isolated only from different patients of the division of pulmonology.

TABLE 1. Isolates of H. influenzae

| Division | No. of strain | | total |
|---|---|---|---|
| | out-patient | in-patient | |
| Pulmonology | 34 (4) | 4 (3) | 38 (7) |
| Other Int. Med. | 6 | 8 | 14 |
| Primary Care | 9 | 0 | 9 |
| Emergency unit | 0 | 6 | 6 |
| Others | 1 | 5 | 6 |
| | 50 (4) | 23 (3) | 73 (7) |
| | 8% | 13% | 9.6% |

( ) : No. of beta-lactamase producing strain

Serotyping by slide agglutination was performed on 31 of the 73 isolates. Fourteen (45%) of these 31 strains were nontypable and 13 (41%) type d, but only 3 strains were type b.

Results of susceptibility of H. influenzae isolates to AMPC and related antibiotics are shown in Figure 1. The minimum inhibitory concentrations (MICs) of AMPC were 0.1 to 6.25 $\mu$g/ml and in 6 of the 7 beta-lactamase producing strains the MICs of AMPC exceeded 0.78 $\mu$g/ml. But MICs of BRL-25,000 ranged from 0.18 to 0.39 $\mu$g/ml, MICs of CZX and CMX was below 0.1 $\mu$g/ml against all of the beta-lactamase producing H. influenzae.

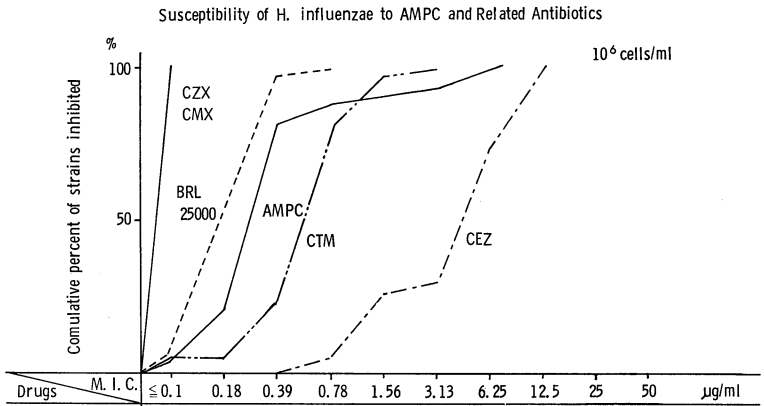
Characteristics of patients with beta-lactamase producing H. influenzae were shown in Table 2.

Six of the 7 patients have had chronic obstructive lung diseases (chronic bronchitis, bronchiectasis, pulmonary emphysema and diffuse panbronchiolitis) and a chemotherapy of penicillins or cephalosporins over a period of 1 to 6 years.

Two H. influenzae were type d, the remaining 4 were nontypable.

Case report : Shown in Figure 2. A 63-year-old female was admitted to Kawasaki Medical School Hospital with fever, cough, purulent sputum and dyspnea. She had been admitted 3 times to our hospital in the last 3 years with same clinical symptoms.

Laboratory findings included the following ; white blood cell count, 11,300/cu mm, erythrocyte sedimentation rate, 70mm/hr, CRP, 8.5 mg/dl, sputum




| Drugs | M. I. C. | ≦0.1 | 0.18 | 0.39 | 0.78 | 1.56 | 3.13 | 6.25 | 12.5 | 25 | 50 | μg/ml |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMPC | (43) | 2 | 7 | 26 | 3* | 1* | 1* | 3** | | | | |
| BRL-25000 | (43) | 3 | 19* | 20** | 1 | | | | | | | |
| CEZ | (38) | | | | 2 | 8* | 9 | 9** | 10** | | | |
| CTM | (38) | 2 | | 7* | 22** | 6 | 1 | | | | | |
| CZX | (38) | 38*** | | | | | | | | | | |
| CMX | (38) | 38*** | | | | | | | | | | |

*: beta-lactamase producing strain

Fig. 1. Susceptibility of H. influenzae to AMPC and Related Antibiotics

TABLE 2. Characteristics of Patients with Beta-Lactamase Producing H. influenzae

| | Age | Sex | Diseae | Bactria | Mic of AMPC | Serotype | Drug | Duration |
|---|---|---|---|---|---|---|---|---|
| 1. M. H. | 61 | M | B. E. | $5\times10^6$ | 3.13 | n. t. | CED | 2 y |
| 2. Y. T. | 52 | M | P. E.<br>B. A.<br>Old T. B. | $1\times10^6$ | 0.78 | d | AMPC | 6 y |
| 3. Z. K. | 77 | M | Lung Ca. | $6\times10^7$ | 6.25 | n. t. | (—) | 0 |
| 4. M. T. | 46 | F | C. B. | (卄) | 6.25 | n. t. | AMPC<br>CEX | 1 y |
| 5. S. S. | 63 | F | D. P. B. | $6\times10^6$ | 6.25 | — | AMPC<br>CEX | 3 y |
| 6. K. O. | 36 | M | B. E. | (卄) | 1.56 | n. t. | AMPC | 2 y |
| 7. K. Y. | 53 | F | B. E. | (卄) | — | d | unknown | 1 y |

B. E. : Bronchiectasis
P. E. : Pulmonary emphysema
B. A. : Branchial asthema
C. B. : Chronic bronchitis
D. P. B. : Diffuse panbronchiolitis

culture showed growth of H. influenzae ($10\times10^6$ cfu/ml).

Immediately she was given an intravenous drip infusion of 2g of TA-058 (new penicillin) which reduced her fever to normal level and the sputum decreased to half volume. However H. influenzae continued positive in sputum

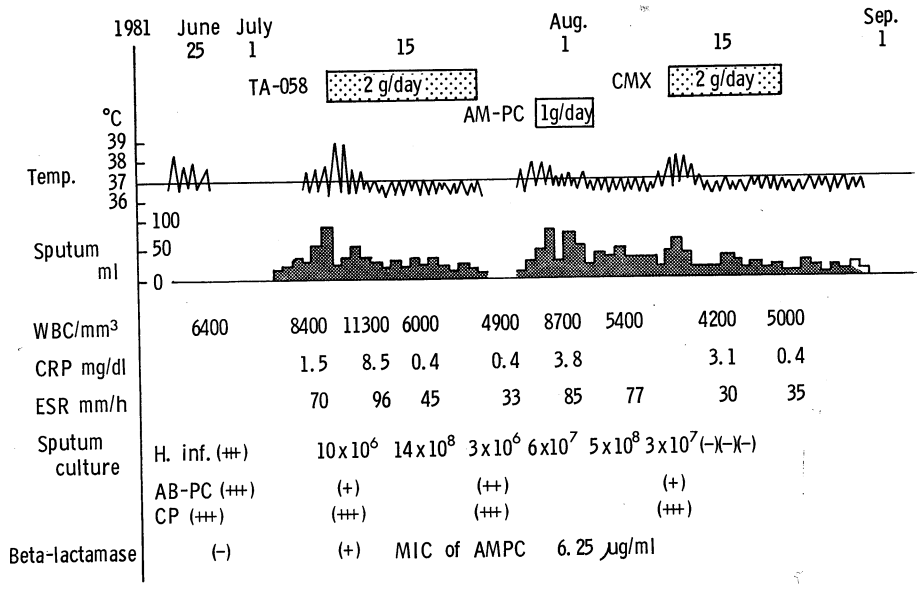



Fig. 2. Case S.S. 63y.o. F. Diffuse panbronchiolitis

culture. This H. influenzae produced beta-lactamase and the MIC of AMPC was 6.25 $\mu$g/ml.

Therefore we decided to use 2g of CMX which is beta-lactamase resistant new cephalosporin. Whereupon there was an instantaneous elimination of H. influenzae in her sputum.

## DISCUSSION

After 1961 ampicillin became widely used for treatment of H. influenzae infections. However, since ampicillin-resistant strains of H. influenzae type b were reported in 1974, an increasing tendency of these strains was recognized, especially in pediatric patients[3,6,7]. Resistance rates ranging from 4.7% to as higher as 15.6% have been reported found by many investigators[7,8].

Our data indicated that 7 (9.6%) of 73 isolates were beta-lactamase producing strains and resistant to ampicillin.

According to many reports resistant H. influenzae were type b in most cases[9,10], but in our findings there is no type b, most of the strains were non-typable.

Against the ampicillin-resistant H. influenzae, the MICs of BRL-25,000[11,12] (AMPC/clavulanic acid which is beta-lactamase inhibitor, 2 : 1) ranged from 0.18 to 0.39 $\mu$g/ml, the MICs of new cephalosporins CZX[13] and CMX[14] were below

0.1 $\mu$g/ml.

Therefore, in conclusion these prove to be extremely valuable in treatment of infections due to beta-lactamase producing ampicillin-resistant H. influenzae.

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