High prevalence of type b β-lactamase-non-producing ampicillin-resistant Haemophilus influenzae in meningitis: the situation in Japan where Hib vaccine has not been introduced

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Objectives: To study yearly changes in resistance and to identify ftsI mutations in β-lactamase-non-producing ampicillin-resistant (BLNAR) and TEM-1 β-lactamase-producing amoxicillin/clavulanic acid-resistant (BLPACR) isolates of Haemophilus influenzae from patients with meningitis.

Methods: Between January 2000 and December 2004, we received 621 isolates of H. influenzae from 285 member institutions of the Nationwide Surveillance Study Group for Bacterial Meningitis. All isolates were analysed by PCR to identify resistance genes and tested for susceptibility to β-lactams. The ftsI gene was sequenced in all BLNAR and BLPACR isolates.

Results: All but four isolates were of serotype b. The isolates could be divided into six classes, namely β-lactamase-non-producing ampicillin-susceptible (25.0%), TEM-1 β-lactamase-producing ampicillin-resistant (11.0%), β-lactamase-non-producing low-level ampicillin-resistant with N526K or R517H substitution in the ftsI gene (30.4%), BLNAR with an S385T substitution together with either N526K or R517H substitution in ftsI (22.2%), BLPACR-I with either a N526K or R517H substitution in ftsI (9.5%) and BLPACR-II with an S385T substitution together with either a N526K or R517H substitution in ftsI (1.9%). The prevalence of BLNAR has increased rapidly, from 5.8% in 2000 to 34.5% in 2004. All BLNAR and BLPACR-II strains were classified into nine subgroups on the basis of substitution patterns in the ftsI gene. The MICs of cephalosporin antibiotics for H. influenzae transformants into which the ftsI genes from BLNAR strains of each of the nine subgroups were introduced increased to varying degrees depending on the mutations.

Conclusions: The results suggest that introduction of H. influenzae type b (Hib) vaccination into infants and children is necessary for the prevention of severe Hib infections in Japan.

Keywords: H. influenzae, BLNAR, PCR

Introduction

The Haemophilus influenzae type b (Hib) vaccine has not been introduced in Japan, and in this country H. influenzae remains a major cause of meningitis in children aged ≥3 months. Surveillance indicates that the incidence of Hib meningitis in Japan is ~10/100 000 in children under 5 years. This is in marked contrast to the low rates in countries where the Hib vaccine has been introduced.2 In the past 6 years, β-lactam-resistant strains of Streptococcus pneumoniae and Hib isolates from patients with meningitis have been rapidly increasing in parallel with their increase in patients with respiratory tract infections (RTIs) in Japan.1,3–5 Of these pathogens, β-lactamase-non-producing ampicillin-resistant (BLNAR)
Table 1. MIC distributions and resistance genes identified by PCR in *H. influenzae* strains isolated in Japan (*n* = 621)

<table>
<thead>
<tr>
<th>Antimicrobial agent and resistance class</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/L)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/L)</th>
<th>MIC range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLNAS</td>
<td>0.25</td>
<td>0.5</td>
<td>0.031–0.5</td>
</tr>
<tr>
<td>BLPAR (TEM-1)</td>
<td>8</td>
<td>16</td>
<td>2–64</td>
</tr>
<tr>
<td>low-BLNAR</td>
<td>1</td>
<td>2</td>
<td>0.5–2</td>
</tr>
<tr>
<td>BLNAR</td>
<td>2</td>
<td>4</td>
<td>1–16</td>
</tr>
<tr>
<td>BLPACR-I</td>
<td>16</td>
<td>32</td>
<td>2–64</td>
</tr>
<tr>
<td>BLPACR-II</td>
<td>32</td>
<td>64</td>
<td>16–64</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLNAS</td>
<td>0.016</td>
<td>0.031</td>
<td>0.004–0.063</td>
</tr>
<tr>
<td>BLPAR (TEM-1)</td>
<td>0.016</td>
<td>0.031</td>
<td>0.008–0.063</td>
</tr>
<tr>
<td>low-BLNAR</td>
<td>0.063</td>
<td>0.125</td>
<td>0.031–0.25</td>
</tr>
<tr>
<td>BLNAR</td>
<td>0.5</td>
<td>1</td>
<td>0.125–2</td>
</tr>
<tr>
<td>BLPACR-I</td>
<td>0.063</td>
<td>0.125</td>
<td>0.016–0.25</td>
</tr>
<tr>
<td>BLPACR-II</td>
<td>0.5</td>
<td>1</td>
<td>0.125–1</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLNAS</td>
<td>0.004</td>
<td>0.008</td>
<td>0.002–0.016</td>
</tr>
<tr>
<td>BLPAR (TEM-1)</td>
<td>0.004</td>
<td>0.008</td>
<td>0.002–0.008</td>
</tr>
<tr>
<td>low-BLNAR</td>
<td>0.016</td>
<td>0.031</td>
<td>0.004–0.125</td>
</tr>
<tr>
<td>BLNAR</td>
<td>0.125</td>
<td>0.25</td>
<td>0.031–0.5</td>
</tr>
<tr>
<td>BLPACR-I</td>
<td>0.016</td>
<td>0.031</td>
<td>0.004–0.125</td>
</tr>
<tr>
<td>BLPACR-II</td>
<td>0.125</td>
<td>0.25</td>
<td>0.031–0.25</td>
</tr>
<tr>
<td>Meropenem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLNAS</td>
<td>0.031</td>
<td>0.063</td>
<td>0.008–0.125</td>
</tr>
<tr>
<td>BLPAR (TEM-1)</td>
<td>0.063</td>
<td>0.063</td>
<td>0.031–0.063</td>
</tr>
<tr>
<td>low-BLNAR</td>
<td>0.125</td>
<td>0.25</td>
<td>0.031–0.5</td>
</tr>
<tr>
<td>BLNAR</td>
<td>0.125</td>
<td>0.25</td>
<td>0.031–0.5</td>
</tr>
<tr>
<td>BLPACR-I</td>
<td>0.125</td>
<td>0.25</td>
<td>0.031–0.5</td>
</tr>
<tr>
<td>BLPACR-II</td>
<td>0.125</td>
<td>0.25</td>
<td>0.063–0.25</td>
</tr>
</tbody>
</table>

Materials and methods

### Strains

A total of 621 *H. influenzae* isolates were collected from clinical laboratories at 285 Japanese medical institutions participating in the NSBM Working Group between January 2000 and December 2004. These institutions were recruited on the basis of their proximity to major metropolitan areas. PCR was immediately performed on all isolates as described below to determine the *fts* mutations, after which production of β-lactamase was confirmed by a nitrocefin test (Showa Chemical Inc., Tokyo, Japan). Isolates were all stored at −80°C for subsequent testing.

The samples were collected after obtaining informed consent from family members in the case of infants and children.

### PCR

PCR was carried out using six sets of primers reported previously,<sup>8,11</sup> the six targets included the 16S rRNA gene (for identification of *H. influenzae*), part of the TEM β-lactamase gene,<sup>12</sup> part of the ROB β-lactamase gene,<sup>13</sup> an N526K amino acid substitution in the *fts* gene, an S385T amino acid substitution in the *fts* gene and a portion of the Hib-specific *capB* locus.<sup>14</sup> PCR cycling conditions were 35 cycles at 94°C for 15 s, at 53°C for 15 s and at 72°C for 15 s.

Isolates suspected of having an R517H substitution on the basis of their susceptibility to ampicillin and cefotaxime were subjected to direct sequencing to detect this substitution because useful primers could not be designed.<sup>8</sup>

### Antibiotic susceptibility

Isolates were tested for susceptibility to ampicillin (Meiji Seika Kaisha, Tokyo, Japan), cefotaxime (Aventis Pharma Ltd, Tokyo, Japan), ceftriaxone (Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) and meropenem (Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan) using an agar dilution method.<sup>15</sup>

### Sequencing

The 1.0 kb DNA fragment of the *fts* gene encoding the PBP3 transpeptidase domain was amplified from the chromosomal DNA of all BLNAR and TEM-1 β-lactamase-producing amoxicillin/clavulanic acid-resistant (BLPACR) strains identified by the PCR method described above and was sequenced by the method reported previously.<sup>10</sup>

### Transformation

An open reading frame that corresponded to the *fts* gene was amplified and transformed into the Rd strain (ATCC 51907) as described previously<sup>10</sup> in a cuvette with a 0.1 cm electrode gap using a MicroPulser<sup>™</sup> electroporation apparatus (Bio-Rad Laboratories, Hercules, CA, USA). The conditions for electroporation were 1.8 kV/cm with time constants of 5.8–5.9 ms. Colonies grown on selective agar plates containing cefotaxime at 0.031 and 0.063 mg/L were selected at random, and the MICs of the β-lactam antibiotics for the colonies were determined. The *fts* gene was also sequenced for several trans-formants to confirm gene transfer.

### Results

#### Age distribution of cases

Among isolates from 621 cases of meningitis, 617 (99.4%) were type b and 4 were non-typeable. The prevalence of *H. influenzae*...
Increase of causative BLNAR in meningitis

Table 2. Changes by year in resistant strains identified by PCR in *H. influenzae* isolated from meningitis patients

<table>
<thead>
<tr>
<th>Year</th>
<th>Resistance class</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BLNAS</td>
<td>24 (34.8)</td>
<td>44 (31.4)</td>
<td>35 (21.1)</td>
<td>26 (20.0)</td>
<td>26 (22.4)</td>
<td>155 (25.0)</td>
</tr>
<tr>
<td></td>
<td>BLPAR</td>
<td>14 (20.3)</td>
<td>19 (13.6)</td>
<td>18 (10.8)</td>
<td>8 (6.2)</td>
<td>9 (7.8)</td>
<td>68 (11.0)</td>
</tr>
<tr>
<td></td>
<td>Low-BLNAR</td>
<td>24 (34.8)</td>
<td>41 (29.3)</td>
<td>52 (31.3)</td>
<td>40 (30.8)</td>
<td>32 (27.6)</td>
<td>189 (30.4)</td>
</tr>
<tr>
<td></td>
<td>BLNAR</td>
<td>4 (5.8)</td>
<td>19 (13.6)</td>
<td>35 (21.1)</td>
<td>40 (30.8)</td>
<td>40 (34.5)</td>
<td>138 (22.2)</td>
</tr>
<tr>
<td></td>
<td>BLPACR-I</td>
<td>3 (4.3)</td>
<td>15 (10.7)</td>
<td>22 (13.3)</td>
<td>12 (9.2)</td>
<td>7 (6.0)</td>
<td>59 (9.5)</td>
</tr>
<tr>
<td></td>
<td>BLPACR-II</td>
<td>0 (0.0)</td>
<td>2 (1.4)</td>
<td>4 (2.4)</td>
<td>4 (3.1)</td>
<td>2 (1.7)</td>
<td>12 (1.9)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>69</td>
<td>140</td>
<td>166</td>
<td>130</td>
<td>116</td>
<td>621 (100.0)</td>
</tr>
</tbody>
</table>

meningitis peaked in patients who were 1 year of age (*n* = 167, 26.9%), and was next highest in patients aged between 7 and 11 months (*n* = 120, 19.3%), and then in patients who were 1–6 months aged (*n* = 106, 17.1%). The total number of isolates from patients aged between 1 and 11 months (*n* = 226, 36.4%) was higher than that in patients who were 1 year of age. Hib meningitis gradually became less prevalent after the age of 2 years and was rare by the age of 5 years. β-Lactam-resistant strains including BLNAR strains were isolated at the same rate in all age groups.

Susceptibility of *H. influenzae* to intravenous agents

Table 1 shows the correlation between resistance genes and susceptibility to the four β-lactams used for empirical treatment of meningitis. The isolates could be grouped into the following six classes: β-lactamase-non-producing ampicillin-susceptible (BLNAS) (*n* = 155; 25.0%); TEM-1 β-lactamase-producing ampicillin-resistant (BLPAR) (*n* = 68; 11.0%); low-BLNAR (*n* = 189; 30.4%); BLNAR (*n* = 138; 22.2%); BLPACR-I with either a N526K or R517H substitution in the *fts*I gene (*n* = 59; 9.5%) and BLPACR-II with an S385T substitution together with either a N526K or R517H substitution in the *fts*I gene (*n* = 12; 1.9%). No ROB-1-positive strains were detected.

The MIC<sub>50</sub> of ampicillin were 1 mg/L for low-BLNAR and 2 mg/L for BLNAR, four to eight times higher than the value of 0.25 mg/L for BLNAS. In contrast, the MIC<sub>50</sub> of cefotaxime and ceftriaxone for the resistant strains were clearly increased. MIC<sub>50</sub> of cefotaxime was 0.063 mg/L for low-BLNAR and 0.5 mg/L for BLNAR, 32 times higher than the value of 0.016 mg/L for BLNAS. Similarly, the MIC<sub>50</sub> of ceftriaxone for BLNAR was 0.125 mg/L, 32 times higher than the value of 0.004 mg/L for BLNAS.

The MICs of meropenem for the resistant strains were affected a little by the *fts*I gene mutations. The MIC<sub>50</sub> of meropenem for low-BLNAR and BLNAR was 0.125 mg/L, four times higher than the value of 0.031 mg/L for BLNAS.

Yearly changes of resistance

Table 2 shows the yearly changes in resistance types classified genotypically among the strains between 2000 and 2004. BLNAR strains were not isolated during half a year in 1999, though the prevalences of low-BLNAR and BLPAR were 29.2% and 31.7%, respectively. However, BLNAR strains were isolated in 2000, since when their prevalence has increased, accounting for 34.5% of isolates in 2004. In contrast, the incidence of BLNAS and BLPAR strains gradually decreased to 22.4% and 7.8%, respectively, in 2004. The incidence rates of low-BLNAR, BLPACR-I and BLPACR-II strains were broadly flat during the 5 years.

Amino acid substitution in the *fts*I gene

Table 3 shows the amino acid substitutions in the *fts*I genes in BLNAR (*n* = 138) and BLPACR-II (*n* = 12) strains classified into nine subgroups on the basis of variation of substitution patterns. BLNAS strain MT196 was taken as the standard ampicillin-susceptible strain. In the BLNAR strains, the six amino acid substitutions shown in Table 3 were important for the development of resistance. Of these substitutions, N526K and R517H were the substitutions which are the base of phenotypic resistance, and three amino acid substitutions were identified surrounding the conserved SSN motif. S385T was common to the II-IV and VI-IX subgroups, and M377I and L389F substitutions were also identified. Strains in subgroup IX with these three substitutions surrounding the SSN motif were the most prevalent.

With regard to the correlation of the number of substitutions and antibiotic susceptibility, the MIC<sub>50</sub> of ampicillin for the BLNAR with three substitutions of S385T, M377I and L389F was only either two or four times higher than those for the low-BLNAR strains. However, the MIC<sub>50</sub> of cefotaxime and ceftriaxone for BLNAR were clearly increased by the additional amino acid substitutions.

Transformation

Ampicillin-susceptible strain Rd was transformed with PCR-amplified *fts*I genes from isolates of each of the nine subgroups of BLNAR strains. The transformation frequency was 1–3/10<sup>7</sup>. As shown in Figure 1, the MICs of cefotaxime for each transformant increased with additional substitutions in the *fts*I gene. The cefotaxime MICs for transformants were as follows: N526K, 0.063 mg/L; S385T + N526K, 0.25 mg/L; M377I + S385T + L389F + N526K, 0.5 mg/L. The cefotaxime MICs for transformants with the four amino acid substitutions of subgroup IX were 64 times higher than the value of 0.008 mg/L for the parent Rd strain. The ampicillin MIC (1 mg/L) for transformants of subgroup IX was only four times higher than the value of 0.25 mg/L for the parent strain Rd. The meropenem MIC for transformants of subgroup IX was 64 times higher than the value of 0.008 mg/L for the parent Rd strain. The correlations between mutation in the *fts*I gene and susceptibility to ampicillin and meropenem were poor.
Discussion

Low-BLNAR strains of *H. influenzae* were first described in the USA in the 1980s, but were not considered an important clinical problem. Instead, TEM-1- and ROB-1-type BLPAR strains have been prevalent to 26% and 10%, respectively, in the USA in 1999, and accordingly, amoxicillin/clavulanic acid has been recommended as a first-line oral antibiotic therapy.

On the other hand, in Japan, TEM-1-type BLPAR appeared in the 1980s and had a prevalence of 15–20% until 1995, after which its prevalence gradually declined to 3% in 1999. The reason for the decrease in prevalence was the development and predominant use of oral third-generation cephalosporin antibiotics which are stable with respect to β-lactamase.

According to retrospective analysis of the *ftsI* gene in *H. influenzae*, low-BLNARs with amino acid substitution

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### Table 3. Amino acid substitutions identified in the *ftsI* genes from BLNAR and BLPACR-II *H. influenzae* strains

<table>
<thead>
<tr>
<th>Resistance class</th>
<th>Subgroup</th>
<th>No. of strains</th>
<th>M377</th>
<th>S385</th>
<th>L389</th>
<th>A502</th>
<th>R517</th>
<th>N526</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLNAS&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.125 0.016 0.004 0.031</td>
</tr>
<tr>
<td>Low-BLNAR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>I 1 (0.7%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>H</td>
<td>–</td>
<td>1    0.063 0.008 0.25</td>
<td></td>
</tr>
<tr>
<td>Low-BLNAR&lt;sup&gt;c&lt;/sup&gt;</td>
<td>II 4 (2.9%)</td>
<td>–</td>
<td>T</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.5 0.063 0.016 0.063</td>
<td></td>
</tr>
<tr>
<td>BLNAR</td>
<td>III 14 (10.1%)</td>
<td>I</td>
<td>T</td>
<td>–</td>
<td>–</td>
<td>H</td>
<td>–</td>
<td>1    0.125 0.063 0.125</td>
<td></td>
</tr>
<tr>
<td>BLNAR</td>
<td>IV 8 (5.8%)</td>
<td>I</td>
<td>T</td>
<td>F</td>
<td>–</td>
<td>–</td>
<td>H</td>
<td>4    1 0.125 0.125</td>
<td></td>
</tr>
<tr>
<td>BLNAR</td>
<td>V 2 (1.4%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>K</td>
<td>2    0.125 0.063 0.25</td>
<td></td>
</tr>
<tr>
<td>BLNAR</td>
<td>VI 11 (8.0%)</td>
<td>–</td>
<td>T</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2    0.25 0.063 0.25</td>
<td></td>
</tr>
<tr>
<td>BLNAR</td>
<td>VII 4 (2.9%)</td>
<td>I</td>
<td>T</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1    0.125 0.031 0.063</td>
<td></td>
</tr>
<tr>
<td>BLNAR</td>
<td>VIII 2 (1.4%)</td>
<td>–</td>
<td>T</td>
<td>F</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>2    0.125 0.063 0.125</td>
<td></td>
</tr>
<tr>
<td>BLNAR</td>
<td>IX 92 (66.7%)</td>
<td>I</td>
<td>T</td>
<td>F</td>
<td>–</td>
<td>–</td>
<td>K</td>
<td>2    0.5 0.125 0.25</td>
<td></td>
</tr>
<tr>
<td>BLPACR-II</td>
<td>II 1</td>
<td>–</td>
<td>T</td>
<td>–</td>
<td>–</td>
<td>H</td>
<td>–</td>
<td>32   1 0.25 0.125</td>
<td></td>
</tr>
<tr>
<td>BLPACR-II</td>
<td>III 1</td>
<td>I</td>
<td>T</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>64   0.125 0.031 0.125</td>
<td></td>
</tr>
<tr>
<td>BLPACR-II</td>
<td>VI 1</td>
<td>–</td>
<td>T</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>K</td>
<td>16   1 0.125 0.063</td>
<td></td>
</tr>
<tr>
<td>BLPACR-II</td>
<td>IX 9</td>
<td>I</td>
<td>T</td>
<td>F</td>
<td>–</td>
<td>–</td>
<td>K</td>
<td>64   0.5 0.125 0.25</td>
<td></td>
</tr>
</tbody>
</table>

AMP, ampicillin; CTX, cefotaxime; CRO, ceftriaxone; MEM, meropenem.

<sup>a</sup>Strain #, MT196.

<sup>b</sup>Strain #, H17.

<sup>c</sup>Strain #, KK01.
R517H or N526K had already emerged in 1985. The low-BLNARs were unlikely to pose clinical problems because their ampicillin and cephalosporin susceptibilities decreased only 2- to 4-fold. BLNAR strains, for which the increases in the MICs of cephalosporins are greater, appeared in 1996 and increased rapidly among isolates from paediatric outpatients with RTIs or acute otitis media. Unfortunately, BLNAR strains of Hib causing meningitis appeared in 2000.1

The antimicrobial activity of cephalosporins against H. influenzae is due to their high affinity for PBP3. Thus, under the selective pressure imposed by oral cephalosporin usage, strains with mutations in the ftsl gene which reduce the binding affinity of PBP3 are selected.

As shown in Table 3, of the nine subgroups identified among the BLNAR and BLPACR-II isolates of H. influenzae, resistant strains belonging to subgroup IX with three amino acid substitutions surrounding the SSN motif and N526K in the ftsl gene were predominantly isolated. These strains in particular showed decreased susceptibility to intravenous cephalosporins, such as cefotaxime and ceftriaxone, which are used for meningitis patients. The results of transformation experiments also supported the fact that the wide distribution of the β-lactam MIC for Hib-type BLNAR reflects the variety of ftsl mutations. These results point to the possible emergence of more highly resistant Hib-type BLNARs possessing new amino acid substitutions in the future.

For clinical efficacy in the treatment of meningitis, it is thought that the concentration of cephalosporins in the CSF needs to be 10–20 times greater than the MIC for the infection strain. At the present time, combination chemotherapy has shifted from ampicillin and cefotaxime to cefotaxime and meropenem or ceftriaxone and meropenem for meningitis patients with BLNAR in Japan. The peak concentrations in CSF achieved following intravenous administration range from 2.6–13.2 mg/L for cefotaxime (at a dosage of 50 mg/kg every 6 h), 7.7 mg/L for ceftriaxone (50 mg/kg every 12 h) and 0.3–2.8 mg/L for meropenem (40 mg/kg every 8 h).19,20 The CSF levels of these agents ranged from 5–26 times the MIC50 of cefotaxime for BLNAR, 62 times the MIC50 of cefotaxime and 3–22 times the MIC50 of meropenem. Monotherapy with these agents for meningitis caused by BLNAR is suspected to be insufficient clinically in some cases.

Data were not shown here, but some meningitis patients have failed monotherapy with cefotaxime or ceftriaxone, and combination therapy was required with β-lactams having different target for PBPs, such as cefotaxime and meropenem or ceftriaxone and meropenem. A questionnaire survey of patients with meningitis indicated that the incidence of severe sequelae was 19.6% (K. Hasegawa, R. Kobayashi, E. Takada, A. Ono, N. Chiba, M. Morozumi, S. Iwata, K. Sunakawa and K. Ubukata, unpublished results). The details will be described in another paper.

Furthermore, according to the NSBM study over 6 years, cases of meningitis caused by BLNAR strains have increased, with the prevalence of these strains being particularly high in patients ≤1 year of age who were immuno immature. An increase in the number of the day nursery children seems to accelerate the increase of infectious diseases caused by these resistant strains. Government approval of the introduction of Hib vaccine should prevent severe Hib infections, including those caused by BLNAR and BLPACR-II strains.

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Transparency declarations

None to declare.

References


