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【 CASE REPORT 】

Bacterial Meningitis Caused by β-lactamase Non-producing Ampicillin-resistant *Haemophilus influenzae* Type f in an Immunocompetent Woman

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Abstract:

We report the case of a 36-year-old previously healthy woman who presented with fever and headache. Blood and cerebrospinal cultures and a bacterial analysis revealed the presence of β-lactamase non-producing ampicillin-resistant *Haemophilus influenzae* type f (Hif) with sequence type 124. Accordingly, the patient was diagnosed with bacterial meningitis with bacteremia caused by Hif. She had normal humoral immunity, and antibiotic therapy rapidly improved her condition. Our case indicates that serotype replacement can occur in Japan and suggests that a certain sequence type causes invasive *Haemophilus influenzae* disease, regardless of host immunity.

Key words: *Haemophilus influenzae* type f, invasive *Haemophilus* disease, bacterial meningitis, β-lactamase non-producing ampicillin-resistant *Haemophilus influenzae*

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Introduction

Haemophilus influenzae strains are classified as typeable (types a-f) or non-encapsulated. Because *H. influenzae* type b (Hib) causes invasive disease in young children, as well as immunocompromised adults, Western countries developed a Hib vaccine in the 1990s. Other countries subsequently did so, including Japan, which established a publicly subsidized Hib vaccination program in 2011. The incidence of Hib infections has decreased due to Hib vaccination; however, the incidence of non-Hib infections has increased (1, 2). *H. influenzae* type f (Hif) is the most common non-b type, and the incidence of Hif infections has risen in the USA, England, Wales, Canada, and Iceland (2-5).

Five cases of invasive disease due to Hif were recently reported in Japan (6-8), all occurred in immunocompetent young children and adults with underlying conditions. Four of the five cases were considered "typical" (i.e., patients were infected with ampicillin-susceptible Hif). We herein report an atypical case of bacterial meningitis with bacteremia caused by β-lactamase non-producing ampicillin-resistant (BLNAR) Hif in an immunocompetent woman.

Case Report

The patient was a 36-year-old woman with no significant medical history. Although she had no apparent sick contacts-including family members-she had a cough, sore throat, and purulent rhinorrhea for approximately two weeks prior to seeking medical attention. The rhinorrhea persisted, whereas the other symptoms improved. She presented to a hospital with fever and headache. At that time computed tomography (CT) of the head showed no abnormalities, and she was discharged. However, her headache worsened, and she was admitted to our hospital the following day.

The patient was unemployed, had reportedly never smoked, and was not pregnant. She lived with her husband and 5-year-old daughter, who had been receiving scheduled Hib vaccinations. A physical examination revealed a body temperature of 37.5°C, a blood pressure of 117/80 mmHg, a heart rate with a sinus rhythm of 75 beats/min, a respiratory rate of 20 breaths/min, and a consciousness level of II-30 on the Japan Coma Scale and E3V5M6 on the Glasgow Coma

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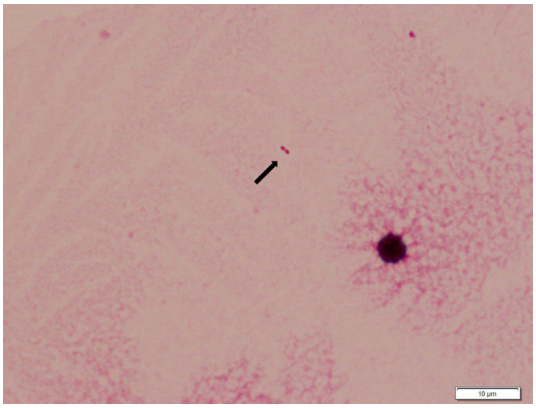

Figure. Gram-stained cerebrospinal fluid smear. Staining revealed Gram-negative bacilli with capsules (*arrows*). *Scale bar* indicates 10 μm.

Scale. Stiffness of the neck was noted.

Blood tests showed leukocytosis [25,400 leukocytes/μL (normal range, 2,700-10,300/μL)], elevated C-reactive protein levels [17.93 mg/dL (normal range, 0-0.30 mg/dL)], and normal glucose levels (141 mg/dL). An examination of the cerebrospinal fluid (CSF) showed a high initial pressure [220 mmH_2O (normal range, 75-170 mmH_2O)], leukocytosis [22,000 leukocytes/3 μL (normal range, 0-15/3 μL)] with abundant polynuclear leukocytes (21,200/3 μL), an elevated protein level [429.7 mg/dL (normal range, 10.0-40.0 mg/dL)], and a low glucose level (<1 mg/dL). A CT scan of the head revealed mucosal thickening in the bilateral maxillary sinus.

We considered the possibility of bacterial meningitis and intravenously administered dexamethasone (26.4 mg per day), ceftriaxone (4 g per day), vancomycin (2 g per day) and ampicillin (12 g per day). Immediately after admission, gram staining of a CSF sample revealed gram-negative bacilli (Figure); thus, dexamethasone, vancomycin, and ampicillin were discontinued. On the 2nd day of hospitalization, the presence of *H. influenzae* was confirmed in blood and CSF cultures. Intravenous antibiotic therapy (ceftriaxone) was continued in accordance with the sensitivity results (Table). Using the nitrocefin disc method (Becton Dickinson and Company, New Jersey, USA), *H. influenzae* isolated from the patient was found to be β-lactamase non-producing, and was consequently identified as BLNAR *H. influenzae*. Based on the culture results, we diagnosed the patient with bacterial meningitis with bacteremia caused by *H. influenzae*. Hif was identified via serotype-specific antisera (Denka Seiken, Tokyo, Japan) and a polymerase chain reaction (9). Multilocus sequence typing revealed that it was sequence type 124 (ST124) (10). Moreover, we investigated the deduced amino acid sequence of the *fstI* gene encoding penicillin-binding protein 3 in the Hif strain isolated from this patient (11). The Hif strain had an amino acid substitution (Asn526Lys), which is reported to be one of the most common amino acid substitutions in the *fstI* gene in BLNAR (11).

Table. Minimal Inhibitory Concentrations (MICs) and Antimicrobial Susceptibility Testing of *Haemophilus influenzae* Type f Isolated from the Patient.

Antimicrobial agent	MIC (μg/mL)	Interpretation*
Ampicillin	4	R
Ampicillin-sulbactam	4	R
Amoxicillin-clavulanate	4	R
Cefaclor	8	R
Cefotaxime	<0.12	S
Ceftriaxone	<0.12	S
Meropenem	0.25	S
Clarithromycin	4	S
Ciprofloxacin	<0.12	S
Trimethoprim-sulfamethoxazole	<0.25	S
Chloramphenicol	<0.5	S
Tetracycline	<0.5	S

*Antimicrobial susceptibility testing was conducted using the microdilution method and judged according to the categories of the Clinical Laboratory Standards Institute.
R: resistant, S: susceptible

The patient's humoral immunity status was further investigated. Her spleen appeared normal on abdominal ultrasound, and her complement and immunoglobulin levels were within the normal ranges. After 14 days of antibiotic therapy, she was discharged from our hospital with no sequelae.

Written informed consent was obtained from the patient for publication of this case report and the accompanying images.

Discussion

We encountered a case of bacterial meningitis due to Hif in an immunocompetent woman. She had persistent purulent rhinorrhea for two weeks before the onset of bacterial meningitis, and mucosal thickening in the bilateral maxillary sinus detected by CT was comparable to acute sinusitis. We therefore considered that acute sinusitis could lead to bacterial meningitis.

The *H. influenzae* strain that causes invasive disease in Japan and other countries with Hib vaccination programs appears to have shifted from Hib to non-b types (1, 2). Due to this shift and consequent changes in clinical features, the number of atypical cases, including those involving immunocompetent patients, will likely increase. ST124 is the most common Hif sequence type in Hif-triggered invasive diseases in some countries (2, 9, 12). In Japan, it was identified in four of the five reported Hif cases (6-8), as well as our own. Accumulating reports on Hif infection suggest that the severity of the infection may be determined by the sequence type, irrespective of the patient's humoral immunity status. Thus, even in the era of Hib vaccines, physicians should consider Hif and other *H. influenzae* serotypes, including non-typeable serotypes, when they encounter patients with severe infections, such as bacterial meningitis.

Invasive Haemophilus disease (IHD) is caused by Hib in

affected children; however, the increasing prevalence of Hif may shift the clinical picture from a childhood disease to a senior disease, and IHD may become associated with a higher mortality rate. Originally-as with Hib-Hif has a high inhibitory effect on an alternative pathway (13), which is considered to be likely to cause IHD. However, although the detailed reasons are unknown, many reports have shown that Hif is more common in elderly people and has a higher mortality rate (2, 14, 15). There have been numerous cases of meningitis caused by Hif in children since it was first reported in 1945 (16), with many cases found in immunocompetent children; the mortality rate in such cases is low. On the other hand, to our knowledge, there have only been three reported cases of meningitis due to Hif in adults (17-19), and all cases had underlying disease; one case was deceased. This suggests that elderly people may be at greater risk due to underlying diseases. Hib infection is typically a childhood infection and the incidence dramatically decreases with vaccination. Thus, elderly patients require early physician attention as the mortality rate of Hif may be higher in elderly people with underlying diseases.

The antibiotic resistance of non-b types of *H. influenzae* is a matter of concern. Little information on the antibiotic resistance of Hif exists, with only a few studies in Japan. In the present case, the Hif strain was found to be BLNAR. The number of BLNAR Hib strains had been increasing prior to the introduction of the Hib vaccine (20), and careful monitoring of the number of ampicillin-resistant Hif, including BLNAR strains, is necessary. In the study by Hoshino *et al.*, one of the three Hif strains isolated from Japanese children required higher concentrations of β lactam antibiotics for growth inhibition than the others; hence, this strain had a lower binding affinity for and susceptibility to these antibiotics (7). In a Canadian study (21), although β-lactamase production was reported to show ampicillin tolerance, BLNAR was not observed.

Although there have been no reports evaluating the association between the incidence of non-b type *H. influenzae* infections and living with children vaccinated for Hib, the introduction of the Hib vaccine in the community may be a predisposing factor for the increasing number of non-type b *H. influenzae* infections. In a study in Italy, the *H. influenzae* strains isolated from the oropharynges of young (<6 years of age) Hib-vaccinated children were predominantly non-typeable *H. influenzae* (11); however, there is no evidence of Hif colonization after Hib vaccination. Because nasopharyngeal colonization changes may occur in children with a complete Hib vaccination status, the adults residing with them may be susceptible to non-b types, such as Hif. Thus, physicians should consider the vaccination history of the patients' family members when treating patients with severe infections. This information may help physicians identify the responsible pathogen and select the most effective antibiotic therapy.

The number of atypical presentations of invasive *H. influenzae* disease, including diseases involving immunocompetent hosts, may be increasing in the Hib vaccination era due to a serotype shift from Hib to non-type b strains. Hence, physicians in Japan should pay careful attention to the surveillance data for invasive diseases involving *H. influenzae*, including the serotypes, sequence types, and antibiotic susceptibility.

The authors state that they have no Conflict of Interest (COI).

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