Recurrent Meningitis Caused by \(\beta\)-Lactamase-Positive Amoxicillin/Clavulanate-Resistant Non-Typeable *Haemophilus influenzae* in a Child with an Inner Ear Malformation: A Case Report

Hyejo Shin 1, Geonju Kim 1, Seung Beom Han 1,2, Dae Chul Jeong 1,2, and Jin Han Kang 1,2

1Department of Pediatrics, College of Medicine, The Catholic University of Korea, Seoul, Korea
2The Vaccine Bio Research Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea

**ABSTRACT**

Infections with *Haemophilus influenzae* type b have been decreasing due to widespread use of conjugate vaccines thereto, and there has been an increasing trend in the relative proportion of invasive infections by non-typeable *H. influenzae* (NTHi). NTHi commonly colonizes the upper respiratory tract and causes recurrent infections of the adjacent organs. There is a rapid development of antibiotic resistance in NTHi strains, and therefore it is important to select appropriate antibiotics for treatment. We report a case of recurrent NTHi meningitis in a 5-year-old girl with a previous history of recurrent otitis media. The patient presented with fever accompanying recurrent vomiting, and \(\beta\)-lactamase-positive amoxicillin/clavulanate-resistant NTHi was isolated in cerebrospinal fluid culture. Antibiotic resistance testing revealed penicillin-binding protein 3 mutation, which is an important emerging mechanism of antibiotic resistance of NTHi. Cystic cochleovestibular malformation was also identified, which may be the predisposing condition for recurrent otitis media, and invasive NTHi infection. Acute symptoms resolved with antibiotic therapy (cefotaxime, 200 mg/kg per day). After surgical revision, the patient has been in good health without recurrence. In children with recurrent respiratory tract infections, or invasive NTHi infection, it is important to consider the presence of underlying diseases and infections due to antibiotic resistant pathogens, in order to select an appropriate antibiotic agent for treatment.

**Keywords:** *Haemophilus influenzae*, Meningitis; Otitis media; Antibiotic resistance

**INTRODUCTION**

*Haemophilus influenzae* is a Gram-negative coccobacillus that can be classified as either one of the encapsulated strains (six distinct serotypes, designated a to f) or as non-typeable *H. influenzae* (NTHi), due to the presence of an external polysaccharide capsule [1]. *H. influenzae*
can be present as a form of nasopharyngeal flora in children of 5 years and younger, and is the predominant nasopharyngeal flora, acting as a source of secondary infection after a respiratory viral infection [2, 3]. The cases of *H. influenzae* type b (Hib) infection, which usually accompanies invasive diseases in children, have dropped considerably since the development and worldwide use of conjugate vaccines. Accordingly, the relative proportion of NTHi compared with Hib in invasive *H. influenzae* infections has increased [4]; therefore, the clinical importance of NTHi infections should be considered. Moreover, the rapid development of antibiotic resistance in NTHi strains has highlighted the need for appropriate antibiotic choices and vaccine development. Here, we report a case of a 5-year-old girl with an inner ear malformation with recurrent meningitis caused by β-lactamase-positive amoxicillin/clavulanate-resistant (BLPACR) NTHi in which the penicillin-binding protein 3 mutation was accompanied by β-lactamase production. We also report a study on the antibiotic resistance mechanisms of the isolated NTHi strain. This report was approved by the Institutional Review Board of Seoul St. Mary’s Hospital (Approval number: KC16ZISE0809).

**CASE REPORT**

A 5-year-old girl was hospitalized with a 3-day history of fever and vomiting. This patient had been repeatedly hospitalized and treated for recurrent otitis media since the age of 1 year. Three years prior, a ventilation tube was inserted in the right ear; she subsequently experienced impaired hearing in the right ear as a complication of the recurrent otitis media. The patient’s vaccination history included four doses each of the 7-valent pneumococcal conjugate vaccine and the Hib conjugate vaccine.

At the time of hospitalization, the patient presented with fever, otalgia, headache, and repeated vomiting. She had a body temperature of 38.8°C, a pulse rate of 100 beats per minute, and a respiratory rate of 28 breaths per minute. Upon examination, her consciousness was clear, but her neck was stiff, and her tympanic membranes were red and bulging. Hematology results were as follows: hemoglobin, 11.6 g/dL; white blood cell (WBC) count, 20,030 /mm$^3$; and platelet count, 345,000 /mm$^3$. The WBC differential count showed a dominance of neutrophils (neutrophils, 78.9%; lymphocytes, 14.9%; and monocytes, 6.0%), while the erythrocyte sedimentation rate and C-reactive protein levels were elevated at 57 mm/hour and 4.38 mg/dL, respectively. Cerebrospinal fluid (CSF) analysis revealed a dominance of neutrophils (neutrophils, 78.9%; lymphocytes, 14.9%; and monocytes, 6.0%), while the erythrocyte sedimentation rate and C-reactive protein levels were elevated at 57 mm/hour and 4.38 mg/dL, respectively. Cerebrospinal fluid (CSF) analysis revealed 2,240 WBCs/mm$^3$, 24 mg/dL glucose, and 53 mg/dL protein, with Gram-negative coccobacilli observed upon Gram staining. Meningitis by *H. influenzae* was suspected, and treatment was started with cefotaxime (200 mg/kg per day), and amikacin (15 mg/kg per day). β-lactamase-positive *H. influenzae* was subsequently identified in CSF culture on the 3rd day of hospitalization. An antibiotic susceptibility test showed resistance to penicillin, ampicillin, and amoxicillin/clavulanate, and susceptibility to cefotaxime. *H. influenzae* was not detected in blood culture tests. Brain magnetic resonance imaging showed chronic otomastoiditis on the right side. Therefore, a temporal bone computed tomography scan was performed to determine involvement of the adjacent organs related to the otitis media; mastoiditis and cystic cochleovestibular malformation were discovered on the right side (Fig. 1).

A polymerase chain reaction test was performed to determine the capsular type of the *H. influenza* identified earlier, and was classified as NTHi. Since the identified strain was resistant
to amoxicillin/clavulanate, in spite of being β-lactamase-positive, a test was performed to identify the other mechanisms of resistance using a method described previously [5]. The results showed multiple amino acid substitutions (Asp350Asn, Met377Ile, Ala502Val, Asn526Lys, Val547Ile, and Asn569Ser) in the penicillin-binding protein 3 (PBP3) encoded region ftsI gene transpeptidase domain, confirming that the NTHi was a BLPACR NTHi with biotype IIb-resistance (Table 1).

The patient’s symptoms improved following treatment in the hospital, and her body temperature returned to normal after 4 days. CSF analysis was repeated on day 7 of hospitalization: WBC counts were normal, and no bacteria were identified in CSF culture. The antibiotics and in-patient treatment were completed on day 16.

However, one month later, the patient was re-hospitalized due to recurrence of the meningitis. The disease recurrence was expected to be caused by the same strain based on identical antibiotic susceptibility results; therefore, neither pulsed-field gel electrophoresis nor nucleic acid sequencing of the identified NTHi were performed. Cefotaxime (200 mg/kg per day) and vancomycin (60 mg/kg per day) were administered, and the patient was hospitalized for 8 days.

Two months later, the patient was admitted again to another hospital due to recurrent meningitis, and ultimately, she underwent surgery for the cystic cochleovestibular malformation (inner auditory canal obliteration). No further recurrence was noted after the surgical intervention.

Table 1. The results of amino acid substitutions in penicillin-binding protein 3

| Group | Amino acid substitution |
|-------|-------------------------|
| IIb   | Asn | Ile | Val | Lys | Ile | Ser |
| Ile   | 348 | 350 | 357 | 368 | 377 | 385 | 389 | 437 | 449 | 502 | 517 | 526 | 530 | 532 | 547 | 551 | 554 | 562 | 569 |
| Asp   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Met   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ile   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

Figure 1. Computed tomography of the temporal bone showing otomastoiditis of the right ear (arrow heads) with cystic cochleovestibular malformation (arrows).
DISCUSSION

NTHi is a common type of nasopharyngeal flora present during infancy, which can invade the mucosa after respiratory infection to cause localized infections, such as otitis media, sinusitis, or bronchitis. In particular, NTHi can exhibit resistance to host clearance mechanisms by forming a biofilm within the middle ear, and can also be resistant to various antibiotics. Conventional culture tests can be negative, even if the NTHi is present [6]. Otitis media caused by NTHi shows a different clinical pattern from otitis media caused by Streptococcus pneumoniae, with lower fever, less ear discharge, frequent recurrence, frequent failure of antibiotic treatment, and common accompaniment by conjunctivitis [2]. Due to these characteristics, NTHi is the most common pathogen to cause recurrent otitis media in children [7], and the chance of developing recurrent otitis media is reportedly four-fold higher in infants aged less than 1 year with NTHi colonization compared to those without [8]. Our patient also had a history of recurrent otitis media that could not be treated with oral antibiotics. Infections with Hib have been decreasing with the introduction of conjugate vaccines, and at the same time, the relative proportion of NTHi compared with Hib increased in terms of invasive H. influenzae infections. The most common forms of invasive infection caused by NTHi are septicemia and meningitis [9]. Unlike Hib-related meningitis, which is transmitted via blood, in NTHi-related meningitis, bacteria are transmitted directly from a local focus [10]. Invasive NTHi infections occur most commonly in infants up to 1 year old, while there is also a higher risk of infection in immunocompromised children, and patients with human immunodeficiency virus infections or chronic respiratory disease [3]. The NTHi meningitis incidence and the risk of recurrence are also higher in children with underlying diseases, such as cerebral injury, CSF leak or ventriculoperitoneal shunt, respiratory infection, or, rarely, cochlear implants or dysplastic internal ear [11]. Such underlying diseases should be suspected, especially in cases of recurrent NTHi meningitis [12]. The patient in this case report also had a cystic cochleovestibular malformation of the inner ear, and developed NTHi meningitis 3 times.

The mechanism of NTHi antibiotic resistance was mediated by β-lactamase [13]. Specifically, the strain that produces β-lactamase and is resistant to ampicillin was first discovered in the United States in 1974, and it is a common strain worldwide [14]. An ampicillin-resistant strain that does not produce β-lactamase (β-lactamase-negative ampicillin-resistance, BLNAR) was first reported in the early 1980s, which complicated the issue of antibiotic resistance in NTHi [15]. Since then, there have been continual reports of antibiotic resistance in NTHi infections. In addition, the proportion of BLNAR H. influenzae strains is gradually increasing in some countries, including Korea and Japan [16]. The mechanism of resistance in the BLNAR strain is due to a mutation in PBP3, in which a substitution of amino acids in the conserved STVK motifs (Ser327-Thr-Val-Lys), SSN (Ser379-Ser-Asn) and KTG (Lys512-Thr-Gly) in the transpeptidase domain of PBP3, causes reduced affinity for β-lactam antibiotics, resulting in antibiotic resistance [17]. Recently, there has also been an increase in infections with the BLPACR strain, in which the PBP3 mutation is accompanied by β-lactamase production, giving the strain resistance even to amoxicillin/clavulanate, and β-lactams [18]. This PBP3 resistance mechanism can be divided into three groups according to the type of mutation in the ftsI gene, which encodes PBP3 [19]. In group I, Arg-517 is mutated to His-517 near the conserved Lys-Thr-Gly motif; in group II, Asn-526 is mutated to Lys-526 (Asn-526-Lys); and in group III, together with the Asn-526-Lys substitution, Met-377 is mutated to Ile-377, Ser-389 to Thr-389, and Leu-389 to Phe-389 near the conserved SSN (Ser-Ser-Asn) motif. Group II is divided into four subgroups: in subgroup Iia, there is no substitution at Ala-502,
and only the Asn-526-Lys substitution is present. In subgroup Ib, there is an additional Ala-502-Val substitution; in subgroup Ic, there is an additional Ala-502-Thr substitution; and in subgroup Id, there is an additional Ile-449-Val substitution. The NTHi strain isolated in our patient exhibited BLPACR and PBP3 mutations (Asp-350-Asn, Met-377-Ile, Ala-502-Val, Asn-526-Lys, Val-547-Ile, and Asn-569), classifying this strain as group II. Specifically, since the strain expresses an Ala-502-Val mutation, this was confirmed as a case of otitis media and meningitis caused by a group Ib BLPACR NTHi strain.

In conclusion, children with underlying diseases, like the patient in this report, are susceptible to invasive NTHi infection, and such susceptibility should be considered during treatment. Meanwhile, it may be possible to find underlying diseases in children with recurrent respiratory tract infections or invasive NTHi infection. Domestically, an increasing number of reports detail NTHi antibiotic resistance due to PBP3 mutations, as well as β-lactamase production [5, 17, 20]. Therefore, in children experiencing recurrent or invasive NTHi infections, it is essential to consider the changes in the patterns of NTHi antibiotic resistance in order to select an appropriate antibiotic agent for treatment.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr. C Park, of The Vaccine Bio Research Institute, for serotyping the H. influenzae isolate.

REFERENCES

1. St Geme JW 3rd, Takala A, Esko E, Falkow S. Evidence for capsule gene sequences among pharyngeal isolates of nontypeable Haemophilus influenzae. J Infect Dis 1994;169:337-42. PUBLMED | CROSSREF
2. Murphy TF, Faden H, Bakaletz LO, Kyd JM, Forsgren A, Campos J, Virji M, Pelton SI. Nontypeable Haemophilus influenzae as a pathogen in children. Pediatr Infect Dis J 2009;28:43-8. PUBLMED | CROSSREF
3. Heath PT, Booy R, Azzopardi HJ, Slack MP, Fogarty J, Moloney AC, Ramsay ME, Moxon ER. Non-type b Haemophilus influenzae disease: clinical and epidemiologic characteristics in the Haemophilus influenzae type b vaccine era. Pediatr Infect Dis J 2001;20:300-5. PUBLMED | CROSSREF
4. Agrawal A, Murphy TF. Haemophilus influenzae infections in the H. influenzae type b conjugate vaccine era. J Clin Microbiol 2011;49:3728-32. PUBLMED | CROSSREF
5. Park C, Kim KH, Shin NY, Byun JH, Kwon EY, Lee JW, Kwon HJ, Choi EY, Lee DG, Sohn WY, Kang JH. Genetic diversity of the fsl gene in β-lactamase-nonproducing ampicillin-resistant and β-lactamase-producing amoxicillin-/clavulanic acid-resistant nasopharyngeal Haemophilus influenzae strains isolated from children in South Korea. Microb Drug Resist 2013;19:224-30. PUBLMED | CROSSREF
6. Jurcisek J, Greiner L, Watanabe H, Zaleski A, Apicella MA, Bakaletz LO. Role of sialic acid and complex carbohydrate biosynthesis in biofilm formation by nontypeable Haemophilus influenzae in the chinchilla middle ear. Infect Immun 2005;73:3210-8. PUBLMED | CROSSREF
7. Leibovitz E, Jacobs MR, Dagan R. Haemophilus influenzae: a significant pathogen in acute otitis media. Pediatr Infect Dis J 2004;23:1142-52. PUBLMED
8. Faden H, Duffy L, Wasielewski R, Wolf J, Krystofik D, Tung Y. Relationship between nasopharyngeal colonization and the development of otitis media in children. J Infect Dis 1997;175:1440-5. PUBLMED | CROSSREF
9. Perdue DG, Bulkow LR, Gellin BG, Davidson M, Petersen KM, Singleton RJ, Parkinson AJ. Invasive *Haemophilus influenzae* disease in Alaskan residents aged 10 years and older before and after infant vaccination programs. JAMA 2000;283:3089-94.

10. Wenger JD, Pierce R, Deaver K, Franklin R, Bosley G, Pigott N, Broome CV. Invasive *Haemophilus influenzae* disease: a population-based evaluation of the role of polysaccharide serotype. *Haemophilus influenzae* study group. J Infect Dis 1992;165(Suppl 1):S34-5.

11. Callanan V, Poje C. Cochlear implantation and meningitis. Int J Pediatr Otorhinolaryngol 2004;68:545-50.

12. Kunze W, Müller L, Kilian M, Schuhmann MU, Baumann L, Handrick W. Recurrent posttraumatic meningitis due to nontypable *Haemophilus influenzae*: case report and review of the literature. Infection 2008;36:74-7.

13. Tristram S, Jacobs MR, Appelbaum PC. Antimicrobial resistance in *Haemophilus influenzae*. Clin Microbiol Rev 2007;20:368-89.

14. Thomas WJ, McReynolds JW, Mock CR, Bailey DW. Letter: Ampicillin-resistant *Haemophilus influenzae* meningitis. Lancet 1974;1:313.

15. Hasegawa K, Kobayashi R, Takada E, Ono A, Chiba N, Morozumi M, Iwata S, Sunakawa K, Ubukata K. Nationwide Surveillance for Bacterial Meningitis. High prevalence of type b b-lactamase-non-producing ampicillin-resistant *Haemophilus influenzae* in meningitis: the situation in Japan where Hib vaccine has not been introduced. J Antimicrob Chemother 2006;57:1077-82.

16. Kim IS, Ki CS, Kim S, Oh WS, Peck KR, Song JH, Lee K, Lee NY. Diversity of ampicillin resistance genes and antimicrobial susceptibility patterns in *Haemophilus influenzae* strains isolated in Korea. Antimicrob Agents Chemother 2007;51:453-60.

17. Dabernat H, Delmas C, Seguy M, Pelissier R, Faucon G, Bennamani S, Pasquier C. Diversity of beta-lactam resistance-conferring amino acid substitutions in penicillin-binding protein 3 of *Haemophilus influenzae*. Antimicrob Agents Chemother 2002;46:2208-18.

18. Barbosa AR, Giufrè M, Cerquetti M, Bajanca-Lavado MP. Polymorphism in ftsI gene and beta-lactam susceptibility in Portuguese *Haemophilus influenzae* strains: clonal dissemination of beta-lactamase-positive isolates with decreased susceptibility to amoxicillin/clavulanic acid. J Antimicrob Chemother 2011;66:788-96.

19. Ubukata K, Shibasaki Y, Yamamoto K, Chiba N, Hasegawa K, Takeuchi Y, Sunakawa K, Inoue M, Konno M. Association of amino acid substitutions in penicillin-binding protein 3 with beta-lactam resistance in beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae*. Antimicrob Agents Chemother 2001;45:1693-9.

20. Bae S, Lee J, Lee J, Kim E, Lee S, Yu J, Kang Y. Antimicrobial resistance in *Haemophilus influenzae* respiratory tract isolates in Korea: results of a nationwide acute respiratory infections surveillance. Antimicrob Agents Chemother 2010;54:65-71.