Comparative Molecular Analysis of *Haemophilus influenzae* Isolates from Young Children with Acute Lower Respiratory Tract Infections and Meningitis in Hanoi, Vietnam

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Received 10 December 2004/Accepted 11 December 2004

Thirty-seven *Haemophilus influenzae* strains from nasopharyngeal swabs (NP) and 44 *H. influenzae* strains from cerebrospinal fluid (CSF) were investigated. Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c, and 1 was type a, whereas all of the 44 isolates from CSF were type b. The MICs of 16 antibiotics for the *H. influenzae* isolates from NP and CSF were similar, and no β-lactamase-negative ampicillin-resistant strain was found. Molecular typing by pulsed-field gel electrophoresis (PFGE) showed that the 37 *H. influenzae* strains from NP had 22 PFGE patterns, with none predominating, and the 44 *H. influenzae* strains from CSF had 9 PFGE patterns, with patterns α (22 isolates) and β (12 isolates) predominating. Our results indicate that two predominant types of *H. influenzae* type b strains have the potential to spread among children with meningitis in Hanoi, Vietnam.

Nontypeable *Haemophilus influenzae* (NTHi) can cause a variety of infections, including otitis media, bronchitis, and pneumonia (7), whereas *H. influenzae* type b (*Hib*) is a common cause of meningitis in children (11). Hib infection rates have been dramatically reduced in countries that have implemented Hib conjugate vaccine programs as part of routine infant immunizations (10). It has also recently been reported that β-lactamase-negative ampicillin (AMP)-resistant (BLNAR) strains have increased in some countries (6, 12), although their global prevalence remains low (4, 5). The aim of our study was to investigate the characteristics of *H. influenzae* among children less than 5 years of age in Vietnam.

Thirty-seven *H. influenzae* strains were isolated from the nasopharyngeal swabs (NP) of 37 children aged 2 to 60 months (mean age, 11 months) who were diagnosed with acute lower respiratory tract infections between 2001 and 2002, and 44 *H. influenzae* strains were isolated from the cerebrospinal fluid (CSF) of 44 children aged 1 to 24 months (mean age, 9 months) who were diagnosed with meningitis between 2002 and 2003, in Hanoi, Vietnam. No patient with an acute lower respiratory tract infection overlapped a patient with meningitis. *H. influenzae* isolates were serotyped by slide agglutination with antisera purchased from Difco Laboratories (Detroit, Mich.), and β-lactamase production was detected by a disk impregnated with nitrocefin (Becton Dickinson, Sparks, Md.). PCR was carried out for *H. influenzae* isolates by using mixed primers (Wakunaga Pharmaceutical Co., Hiroshima, Japan), as described previously (3). MICs were determined by the agar dilution method according to the NCCLS guidelines (8). The susceptibilities of 81 *H. influenzae* isolates to the following 16 antibiotics were tested: penicillin G (Meiji Seika Kaisha, Tokyo, Japan), AMP (Meiji Seika Kaisha), amoxicillin-clavulanic acid (AMC) (GlaxoSmithKline K.K., Tokyo, Japan), cefadroxil (Taiyo Yuhlin Co., Nagoya, Japan), cefuroxime (Sankyo Co., Tokyo, Japan), ceftriaxone (Chugai Pharmaceutical Co., Tokyo, Japan), cefotaxime (Aventis Pharma, Tokyo, Japan), imipenem (Banyu Pharmaceutical Co., Tokyo, Japan), minocycline [Lederle (Japan), Tokyo, Japan], chloramphenicol (Sankyo Co.), clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan), erythromycin (Dainippon Pharmaceutical Co., Osaka, Japan), gentamicin (Schering-Plough K.K., Osaka, Japan), levofloxacin (Daiichi Pharmaceutical Co., Tokyo, Japan), norfloxacin (Kyorin Pharmaceutical Co., Tokyo, Japan), and sulfamethoxazole-trimethoprim (Shionogi & Co., Osaka, Japan). After digestion with SmaI (Takara Shuzo Co., Shiga, Japan), pulsed-field gel electrophoresis (PFGE) was performed on the 37 *H. influenzae* isolates from the NP and the 44 *H. influenzae* isolates from the CSF, as described previously (16), and the interpretation of PFGE patterns was based on the criteria described by Tenover et al. (13).

Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c, and 1 was type a, whereas the 44 isolates from CSF were all type b. Twenty-six strains (70.3%) from NP and 23 strains (52.3%) from CSF were β-lactamase producing, and the remaining strains were β-lactamase negative by the nitrocefin disk assay. PCR analysis to identify the resistance genes indicated that 25 strains from NP and 21 strains from CSF were TEM-1-type β-lactamase gene; 11 strains from NP and 22 strains from CSF were β-lactamase-negative AMP-susceptible isolates, all of which lacked all resistance genes; and 1 strain each from NP and CSF were TEM-1 β-lactamase-producing AMP-resistant isolates.
which had the TEM-1-type β-lactamase gene and the ftsI gene with the same substitution as the low-BLNAR strains. Although all isolates from NP which had the TEM-1-type β-lactamase gene were β-lactamase producing by the nitrocefin disk assay, one isolate from CSF which had the TEM-1-type β-lactamase gene was β-lactamase negative and two isolates from CSF which did not have the TEM-1-type β-lactamase gene were β-lactamase producing by the nitrocefin disk assay. No BLNAR strain was found. Table 1 shows the MIC range, the MICs at which 50% of isolates were inhibited (MIC$_{50}$), and the MIC$_{90}$ of 16 antibiotics for 37 $H$. influenzae strains isolated from nasopharyngeal swabs and cerebrospinal fluid from children in Vietnam. Although the MICs of the $H$. influenzae isolates from NP against penicillin G and AMP appear to be higher than those from CSF, the antimicrobial susceptibilities of the $H$. influenzae isolates from NP and CSF were similar. Molecular typing by pulsed-field gel electrophoresis (PFGE) showed that the 37 $H$. influenzae strains from NP had 22 PFGE patterns (A to V), without any predominant pattern (Fig. 1). The PFGE patterns of $H$. influenzae types a, b, and c were different from those of NTHi. Four isolates of type b had two PFGE patterns (I and K), and two isolates of type c had two PFGE patterns (H and Q). Forty-four $H$. influenzae strains from CSF had nine PFGE patterns (a to i), with patterns α (22 isolates) and β (12 isolates) predominating. The PFGE patterns of 4 $H$. influenzae type b strains from NP were quite different from those of the 44 $H$. influenzae type b strains from CSF (Fig. 2).

In conclusion, our results demonstrate that BLNAR strains are not prevalent and that two predominant types of Hib in the upper respiratory tract because of their low immunity (16), and subsequent colonization can become a risk factor for invasive diseases caused by $H$. influenzae (2, 11). Since it has recently been reported that BLNAR NTHi and Hib have increased in some countries (3, 6, 12), the primary objective of this study was to investigate such resistant strains among children in Vietnam. In fact, no BLNAR strains were found in either NP or CSF, although more than half the isolates were β-lactamase producing and had the TEM-1-type β-lactamase gene. Hib remains the major cause of meningitis after the introduction of Hib vaccine in many advanced nations, because that vaccine is not usually available in Vietnam (14). Therefore, a secondary objective of this study was to examine the transmission route of $H$. influenzae. It has recently been reported that children can acquire $H$. influenzae at day care centers (9, 16) or from their parents at home (15). Our PFGE studies showed that NTHi did not have dominant genetic patterns but that Hib had two dominant genetic patterns. The results provide evidence to show that at least two types of Hib strains are spreading horizontally among children with meningitis in Vietnam. The Hib conjugate vaccine appears to be effective, not only for the prevention of invasive diseases, but also for the reduction of nasopharyngeal carriage in young children (1, 10).

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In conclusion, our results demonstrate that BLNAR strains are not prevalent and that two predominant types of Hib
strains have the potential for spreading among children with meningitis in Hanoi, Vietnam. Therefore, the introduction of the Hib conjugate vaccine for young children should be considered in order to prevent invasive diseases caused by Hib.

We thank Akihiro Wada (Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University), Chieko Shimauchi (Miyazaki Prefectural Nursing University), and Matsuhisa Inoue (Kitasato University School of Medicine) for help with PCR studies. We also thank Yoko Takashima and Naoko Kitajima (Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University) for help with completion of the PFGE studies. We thank the Hib conjugate vaccine for young children should be considered in order to prevent invasive diseases caused by Hib.

This study was supported by the Core University Program, sponsored by the Japan Society for the Promotion of Science (JSPS).

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