

Nasopharyngeal carriage and antimicrobial susceptibility profile of *Haemophilus influenzae* among patients infected with HIV in Jakarta, Indonesia

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Abstract

In this study, the prevalence of nasopharyngeal carriage and the antimicrobial susceptibility profile of *Haemophilus influenzae* were investigated in children and adults with HIV infection in Jakarta, Indonesia. Thirty-four *H. influenzae* isolates were identified in the children ($n=16/90$; 18%) and adults ($n=18/200$; 9%) infected with HIV. All isolates were nontypeable *H. influenzae* and were less susceptible to ampicillin (62%) and trimethoprim/sulfamethoxazole (41%). In this study, the *H. influenzae* strains carried by patients infected with HIV were dominated by non-capsulated types.

INTRODUCTION

Haemophilus influenzae is a Gram-negative coccobacillus with pleiomorphic morphology and is a constituent of the normal upper respiratory tract flora [1]. *H. influenzae* serotype b (Hib) is reported to be an invasive serotype causing at least 30% of fatal infections such as meningitis and pneumonia in many countries [1, 2]. The current widespread use of Hib conjugate vaccines has nearly eradicated invasive Hib diseases in children in many countries; however, this achievement was accompanied by a shift in capsular serotypes, with nontypeable *H. influenzae* (NTHi) strains replacing type b strains [3].

In Indonesia, the Hib conjugate vaccine has been implemented for some decades, but the epidemiological data on *H. influenzae* are limited. One available epidemiological datum was the prevalence of *H. influenzae* carriage in healthy children aged 0–2 years in Lombok with a prevalence of 32% [4]. Recently, Dunne *et al.* reported that the carriage prevalence of *H. influenzae* was 27.5% among 302 healthy children aged 12–24 months in the Bandung, Central Lombok and Padang regions [5]. In this study, we investigated the carriage prevalence of *H. influenzae* in patients with HIV infection in Jakarta, Indonesia

METHODS

The nasopharyngeal swab specimens used in this study were archived specimens from previous pneumococcal carriage studies [6, 7]. There were a total of 90 and 200 nasopharyngeal swab specimens from children (mean age 69 months) and adults (mean age 35 years), respectively, with HIV infection, in Jakarta, Indonesia, in 2012. *H. influenzae* was isolated by inoculating 100 μ l of nasopharyngeal swab specimens onto chocolate agar media supplemented with bacitracin (20 U ml⁻¹) and incubated at 37°C with 5% CO₂ for 18–24 h. All suspected isolates were identified by Gram staining, oxidase test and XV factor-dependent test [8] and were confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [9] and real-time polymerase chain reaction (RT-PCR) for *H. influenzae* gene-encoding protein D (*hpd*) [8]. Serotyping was done by RT-PCR as described previously [8]. Antimicrobial susceptibility test was performed by the disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) standard.

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Keywords: HIV-infected individual; nasopharyngeal carriage; nontypeable *Haemophilus influenzae*.

Abbreviations: Hib, *H. influenzae* serotype b; HIV, human immunodeficiency virus; MALDI TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NTHi, nontypeable *Haemophilus influenzae*; PCR, polymerase chain reaction.

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Table 1. Antimicrobial susceptibility profile of *H. influenzae* isolated from the nasopharynx of HIV-infected individuals

Antimicrobial agent	All isolates (n=34), (%)	Children (n=16), (%)	Adult (n=18), (%)
Trimethoprim/sulphamethoxazole	14 (41)	4 (25)	10 (56)
Cefotaxime	33 (97)	15 (94)	18 (100)
Ampicillin	21 (62)	7 (44)	14 (78)
Chloramphenicol	27 (79)	11 (69)	16 (89)
Azithromycin	34 (100)	16 (100)	18 (100)
Levofloxacin	34 (100)	16 (100)	18 (100)

RESULTS

Thirty-four *H. influenzae* strains were isolated from 290 nasopharyngeal swab specimens (12%), with 16 and 18 strains being isolated from children (18%) adults (9%), respectively. All isolates were identified as Gram-negative coccobacilli, oxidize-positive, and were dependent on factors X and V with *hpd* being positive. The results from MALDI-TOF MS revealed *H. influenzae* as having the highest identification score in all 34 isolates. All isolates were found to be defined as NTHi by the real-time PCR method.

All isolates were still susceptible to levofloxacin and azithromycin, 97% were susceptible to cefotaxime, 79% were susceptible to chloramphenicol and 62% were susceptible to ampicillin (Table 1). NTHi isolates were found to be less susceptible to trimethoprim/sulfamethoxazole (41%). Children with HIV infection were observed to have less antimicrobial susceptibility than adults with HIV infection to trimethoprim/sulfamethoxazole (25 vs 56%), ampicillin (44 vs 78%) and chloramphenicol (69 vs 89%) (Table 1).

DISCUSSION

In this study, the prevalence of carriage in children with HIV infection (18%) was higher than that in a study conducted in Tanzania with a prevalence of carriage of 14% in infants exposed to HIV [10]. In India, the use of Hib conjugate vaccine caused a decrease in the prevalence of colonization from 13.8 to 1.8% in children, 2–15 years of age, infected with HIV [11]. However, our findings in children were lower than those in a study conducted in Zambia with a prevalence of colonization of 29% [12]. Children infected with HIV were reported to have a higher prevalence of colonization by *H. influenzae* than children not infected with HIV [13].

We observed that all strains were non-encapsulated types of NTHi. These results were slightly different from those of the studies conducted in 1997 in Lombok, Indonesia, which succeeded in isolating 22 *H. influenzae* serotype b isolates, although the majority of strains were NTHi [4]. The absence of serotype b could have been due to several factors affecting the serotype, including the use of Hib-containing pentavalent

vaccine that had become part of the routine immunization programme in Indonesia since 2013 [14].

Resistance to trimethoprim/sulfamethoxazole was more dominant in the children group because this antimicrobial drug was generally used for prophylaxis in children [12, 15]. In this study, 62% of the NTHi isolates were susceptible to ampicillin. This was much lower than in a study conducted in 1997 in Lombok Island, where all of the *H. influenzae* isolates (155 isolates) were susceptible to ampicillin [4].

Since the implementation of Hib vaccination, we discovered that the serotype of *H. influenzae* circulating in children and adults was predominantly NTHi. Given the high prevalence of NTHi found in the high-risk group (HIV group) and the reduced susceptibility profile for antibiotics, this study can be considered for NTHi vaccine development and implementation to reduce the risk of *H. influenzae* infection cases in Indonesia. In conclusion, the prevalence of *H. influenzae* carriage in children and adults infected with HIV in Jakarta, Indonesia was 12% and was dominated by the non-encapsulated type. The *H. influenzae* isolates in this study were less susceptible to trimethoprim/sulfamethoxazole.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

The studies have been reviewed and approved by the Ethical Committee of Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. The participants (adults) signed informed consent forms and for the participants under 16 years old, an informed parental/guardian (grandfather/grandmother) permission form was completed prior to enrolment.

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