Factors Associated with Oropharyngeal Carrier of *Haemophilus influenzae* and Antimicrobial Resistance in Healthy Children Attending Day-care Center of a Health Promotion Hospital

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ABSTRACT

A cross sectional study was performed in the children attending a day-care center of Health Promotion Hospital to evaluate the carriage rate and factors associated with the *Haemophilus influenzae* oropharyngeal colonization and antimicrobial susceptibility of *H. influenza*. The samples were 189 children, 95 boys and 94 girls. The data were collected by self-administered questionnaire, the secondary data by reviewing the history folder recorded by the day care, physical examination and oropharyngeal swab during July–November 2007. Data were analyzed by frequency, percentage, Chi-square test, and Multiple logistic regression. The results showed that 44.4% of the children were the oropharyngeal carrier of *H. influenza* and 1.1% of these were *H. influenzae* type b. Factors associated with *H. influenzae* carrier with statistical significance were the children's age less than 3 years, history of premature, birth weight less than 2,500 grams, the present of more than 2 children aged less than 10 years at home, family members of more than 5 persons, maternal occupation of employee or government officer, extended family, and the children not received *H. influenzae* type b (Hib) vaccine. The factors influencing the oropharyngeal carrier of *H. influenzae* were history of preterm labor, and the number of over 2 children aged below 10 years living in the house. For antimicrobial susceptibility test results 68.5% of *H. influenzae* were resistant to cotrimoxazole, 31.5% to ampicillin, and 1.1% to cefotaxime. Therefore, the family and child care organizations should have the plan to prevent the spreading of disease and reduce the chances *of H. influenzae* carrier in children since pregnancy by preventing preterm labor, low birth weight and health care in the family with several members particularly in the family which have the children aged below 10 years, as well as giving the vaccine to reduce the risks of carrier, transmission prevention, and the usage of appropriate antibiotics.

Key words: Oropharyngeal carrier, *H. influenzae,* healthy children, Day-care Center, antimicrobial resistance

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Introduction

The preschool children has high rate of development and learning, it is the time to establish the appropriate growth and development in all aspects, as the healthy adults are the result of healthy children. The respiratory tract infection is the important cause of the illness and death in the children particularly in the developing countries. The common causative organism is *Haemophilus influenzae,* a bacteria found in the membrane of the respiratory tract of the normal child without causing any disease[1]. *H. influenzae* has several strains, but the strain that causes severe disease is *Haemophilus influenzae* type b (Hib). Man is only natural reservoir host and was colonized in the respiratory tract by which 60-90% of children are non-typable strain of *H. influenzae* carrier, and 2-5% are the carrier of *H. influenzae* type b which causes the disease in children aged 2 months to 5 years but more common in aged less than 2 years as a cause of meningitis, pneumonia, arthritis, and septicemia[2]. *H. influenzae* can spread to others by close contact with the carrier children, especially where children live together like in day-care center, congested community, and crowded place. The incidence of *H. influenzae* type b meningitis before the launching of vaccine in United State was 40-56 cases : 100,000 children aged under 5 years per year[3], but after vaccine usage, the incidence were 98% decreased and also found this effect in many countries[4]. In Thailand, *H. influenzae* type b is the most common bacterial meningitis (42-44%)[5] and from the study of Department of Disease Control in 2000-2001 reported the incidence of *H. influenzae* type b meningitis by 3.8 cases : 100,000 children aged under 5 years per year[6]. However the prevention of *H. influenzae* type b by vaccination is the option and not widely use.

In the treatment of patients infected with *H. influenzae* type b is extremely important to give antibiotics due to the rapid progress and high morbidity and mortality. Recently, *H. influenzae* type b resist to penicillin group by increasing enzyme β-lactamase production. In the United States reported the penicillin resistant almost 50%[7]. In Thailand, the study of National Child Health Great Queen (Children's Hospital) found 43% of *H. influenzae* type b resisted to ampicillin[8] while penicillin group was widely used in health care centers and drugstores. As mention above, the *H. influenzae* infection in the children is still the important problem particularly in the places where many children living together. So, this study aimed to find out the prevalence of oropharyngeal carrier of *H. influenzae* in children, factors associated with the carrier stage, and the antimicrobial resistance that will lead to the appropriate prevention and treatment.

Materials and Methods

The cross-sectional study was performed in every children aged 1½ - 5 years attending the day care center of Health Promotion Center Region 1 with the amount of 189 children between July to November, 2007 under the consent of their parents. The study tools consisted of 3 parts. Part I was the self-administered questionnaire constructed by the researchers for the main caregiver composed of 17 items about the children and 16 items about the parents and family. Part II was the secondary data about the maternal history of pregnancy and child's vaccination collected by the researchers by reviewing the child's history folder recorded by the day-care. Part III was the general physical examination by 2 pediatricians, and the specimens were collected by oropharyngeal swab and transferred in Stuart transport media under the temperature control of 4 degree Celsius, and isolated the existing bacteria in microbiological laboratory within 48 hours. Isolation was done by inoculated the specimen from Stuart transport media into chocolate agar and blood agar plate then incubated in CO_2 incubator jar at 37 degree Celsius for 1 night. The *H. influenzae* was isolated by considered the typical colony grew in chocolate agar but no growth in blood agar, Gram stain, growth factor dependent test by applying factor X, V, and XV. The *H. influenzae* type b was identified by slide agglutination test, using the antiserum of *H. influenzae* type b.

Antimicrobial susceptibility for *H. influenzae* was tested by the disc diffusion method and interpreted the results by comparison with the standard of National Committee for Clinical Laboratory Standard (NCCLS)[9]. The antimicrobials tested were ampicillin, cotrimoxazole, chloramphenicol, and cefotaxime.

Data were analyzed by frequency and percentage to describe the prevalence of *H. influenzae* carrier and antimicrobial susceptibility, Chi-square was used to find the associated factors, and Multiple logistic regression to find the factors influencing the carrier stage of *H. influenzae.*

Ethical consideration

The research was approved by the Research Ethical Committee, Faculty of Public Health, Mahidol University, with a documentary proof number of MUPH 2006-116.

Results

The result showed the 189 children were aged between 33 to 43 months with the average of 30 months, 50.3% were boys and 49.7% were girls, all children were healthy. The data giver were mothers (68.3%), fathers (20.1%), and others family members (11.6%). The main caregiver were mother (66.1%), grandparent/aunt/uncle (19.0%), both father and mother (10.6%), and father (4.3%). Majority of children had appropriate weight for age (66.7%), height for age (70.9%), and weight for height (69.3%). Almost all children (99.5%) were fully immunized with 39.7% received additional vaccine in which 60.0% of them received vaccine for prevention of *Haemophilus influenzae* type b meningitis (Hib vaccine) and only 1.3% received pneumococcal vaccine (IPD). For the others 38.7% received other vaccines (hepatitis A and varicella vaccine).

By the history at birth, most of mothers (97.4%) had complete 4 antenatal care visits, with 84.7% were term delivery, 10.6% with premature labor, and 4.7% postterm labor. For mode of delivery, 48.1% were normal labor, 51.9% delivered by other means (cesarian section, forcep extraction, and vacuum extraction). Most of children (84.1%) had normal weight at birth, 9.5% with low birth weight, and 6.3% overweight at birth. About 12.2% of children were not breastfed, 44.4% had less than 3 months breastfed, and 43.4% breastfed longer than 3 months.

For the number of children younger than 10 years old in the family, 40.2% had only one child, and 59.8% had two of more children. For the family type, 54.5% were extended family, and 45.5% were nuclear family, while 52.9% of all had more than 5 family members. The occupation of the father, 77.3% were government officer or employee, 15.3% had private business. The occupation of the mother, 55.6% were government officer or employee, and 19.0% had private business. Smoking in the home found up to 45.5%. The general data of children and family is shown in Table1.

Table 1 Number and percentage of personal factors and family factors (n = 189)

Variables	n	%
Sex		
Male	95	50.3
Female	94	49.7
Age		
≤ 24 months	7	3.7
25 to 36 months	160	84.7
> 36 months	22	11.6
Term of labor		
Preterm labor	20	10.6
Term labor	160	84.7
Postterm labor	9	4.7
Birth weight		
Low birth weight (<2,500 g)	18	9.5
Normal birth weight (2,500 to 3,800 g)	159	84.1
Over birth weight (> 3,800 g)	12	6.4
Additional vaccine (n = 75)		
Hib vaccine	45	60.0
IPD vaccine	1	1.3
Other vaccines (HAV, varicella vaccine)	29	38.7
Father's occupation		
Trade/private business	29	15.3
Government officer/employee	146	77.3
Others	14	7.4
Mother's occupation		
Trade/private business	36	19.0
Government officer/employee	105	55.6
Others	48	25.4
Family type		
Nuclear family	86.	45.5
Extended family	103	54.5
Number of children aged below 10 years at home		
1 child	76	40.2
2 or more children	113	59.8
Number of family members		
3 - 4 members	39	20.6
≥ 5 members	150	79.4

Prevalence of *H. influenzae* carrier and factors associated with oropharyngeal carrier of *H. influenzae*

By culture from the oropharyngeal swab found 92 strains of *H.influenzae* localized on the throat of 84 children (44.4%) and found 1.1% were *H. influenzaeb type B* carrier. Factors associated with oropharyngeal carrier of *H. influenzae* with statistical significance were age under 36 months (p = 0.029), history of premature delivery (p = 0.004), birth weight less than 2,500 grams (p = 0.013), the number of 2 or more children aged below 10 years at home (p = 0.020), family members of more than 5 persons (p = 0.027), mothers were government officer or employee (p = 0.031), extended family type (p = 0.034), history of receiving *H. influenzae type* b vaccine (p = 0.039). (Table 2)

Table 2 Factors associated with oropharyngeal carrier of *H. influenzae* in children (n = 189)

Variables	Carrier stage of *H. influenzae*		*p-value***
	Carrier Number (%)	Non carrier Number (%)	
Age (months)			0.029*
≤ 36	79 (47.3)	88 (52.7)	
> 36	5 (22.7)	17 (77.3)	
Term of labor			0.004*
Term/postterm labor	69 (40.8)	100 (59.2)	
Preterm labor	15 (75.0)	5 (25.0)	
Birth weight			0.013*
≥ 2,500 grams	71 (41.5)	100 (58.5)	
< 2,500 grams	13 (72.2)	5 (27.8)	
Number of children aged below 10 years at home			0.020*
1 child	26 (34.2)	50 (65.8)	
≥ 2 children	58 (51.3)	55 (48.7)	
Number of family members			0.027*
3 - 4 members	32 (36.0)	57 (64.0)	
≥ 5 members	52 (52.0)	48 (48.0)	
Mother's occupation			0.031*
Government officer/employee	54 (51.4)	51 (48.6)	
Trade/private business/others	30 (35.7)	54 (64.3)	
Family type			0.034*
Nuclear family	31 (36.0)	55 (64.0)	
Extended family	53 (51.5)	50 (48.5)	
Hib vaccination			0.039*
Yes	26 (57.8)	19 (42.2)	
No	58 (40.3)	86 (59.7)	

* significant at $p < 0.05$

** Test by Chi-square test

Influencing factors for oropharyngeal carrier of *H. influenzae* in children

Factors statistical significance associated with the *H. influenzae* carriage were selected and analyzed by Multiple logistic regression revealed that the history of preterm labor, and the number of 2 or more children aged below 10 years at home had an influence on *H. influenzae* carriage p = 0.043, OR = 3.397 (1.042 – 11.073 and p = 0.041, OR = 1.990 (1.028 – 3.852). The factors which had no influence on the carrier stage of *H. influenzae* were age of the children, birth weight, number of family members, mother's occupation, family type, and history of Hib vaccination. (Table 3)

Table 3 Factors influencing the oropharyngeal carrier of *H. influenzae* in children (n = 189)

Factors	B	OR	95% CI
Age[1]			
≤ 36 months	-1.064	0.345	0.112-1.068
Term of labor[2]			
Preterm labor	1.223*	3.397	1.042-11.073
Birth weight[3]			
< 2,500 grams	0.917	2.501	0.772-8.099
Number of children aged below 10 years at home[4]			
≥ 2 children	0.688*	1.990	1.028-3.852
Number of family members[5]			
≥ 5 members	0.274	1.315	0.515-3.356
Mother's occupation[6]			
Government official/employee	0.571	1.771	0.928-3.377
Family type[7]			
Extended family	0.639	1.895	0.734-4.889
Hib vaccination[8]			
Received Hib vaccine	0.669	1.952	0.930-4.099
-2 Log likelihood -	225.60		
p-value =	< 0.001		
n	189		

* p-value < 0.05; OR : Odds ratio; 95% CI: 95% confidence interval; 0 = not carrier; 1 = carrier

Reference groups: 1 = children aged over 36 months, 2 = term/postterm labor, 3 = birth weight ≥ 2,500 grams, 4 = 1 child aged below 10 years at home, 5 = 4 or less family members, 6 = mother's occupation of trade/private business/others, 7 = nuclear family, 8 = did not receive Hib vaccine

Antimicrobial resistant

The 92 isolates of *H. influenzae* cultured from 189 children were highest resistant to cotrimoxazole (68.5%) followed by ampicillin (31.5%), chloramphenicol (6.5%), and cefotaxime (1.1%). (Table 4)

Table 4 Antimicrobial susceptibility of *H. influenzae* (n = 92)

Antimicrobial	Susceptible n (%)	Intermediate susceptible n (%)	Resistant n (%)
Cotrimoxazole	29 (31.5)	0 (0)	63 (68.5)
Ampicillin	61 (66.3)	2 (2.2)	29 (31.5)
Chloramphenicol	86 (93.5)	0 (0)	6 (6.5)
Cefotaxime	91 (98.9)	0 (0)	1 (1.1)

Discussion

The prevalence of oropharyngeal carrier of *H. influenzae* in children was 44.4% compared with many studies which found the carrier rate from 11.9%[10] to 72.0%[11-13], and the study by Hashida K et al[14] in 2008 who studied the infant to 6 years old children in daycare center and found 47.7% carrier stage while the study in Thailand by Olsen SJ et al[16] who studied in out patient clinic of respiratory tract infection in both children and adults found 36.0% were carrier of *H. influenzae* and 6.4% of *H. influenzae* type b (Hib) while the present study found 1 case of Hib (1.1%). It is notable that the present study found the higher rate of *H.influenzae* carrier than the studied in patients of respiratory system. This may due to population of the present study were the children age younger than 5 years which normally had higher carrier stage than other age groups. Comparing with the study of Pitikultang S et al[15] also found the higher prevalence of *Streptococcus pneumoniae* nasopharyngeal carriage in 3 years old children. For Hib carrier found only 1.1% which less than the studied of Olsen SJ et al[16], and Simasathien S et al[17] in orphanage home in Bangkok that found 9-17% carrier stage due to the different in the condition of population groups.

Factors associated with the carriage stage of *H. influenzae* were age of children less than 36 months, history of preterm labor, low birth weight in which these were considered as high risk groups due to their low and immature immunity. The present of more than 2 children below 10 years living together in the house, there were more than 5 members in the family, and the extended family type were associated with the carrier stage which congruent with many studies which stated that the carrier stage related to the chance of exposure, the crowed family or the household with many children living together[18]. History of Hib meningitis vaccination also associated with the carrier stage which congruent with many studies which revealed that Hib vaccination could reduce the oropharyngeal carrier stage of *H. influenzae*[19]. The study of Wang S et al[20] in healthy children aged under 5 years found 52.6% of the children received Hib vaccine and all of them were not found *H. influenzae* colonization in their throat, but in the contrary those without Hib vaccination had 1.27% *H. influenzae* carrier.

Factors influencing the oropharyngeal carriage stage of *H. influenzae* were premature labor and the present of more than 2 children aged below 10 years living together in the house, this could explain that premature baby was the high risk group especially in the first year of life, they might adapt and grow rapidly to survive and live with the changing environmental so they had higher chance of infected than normal baby. The present of many children in the house would have the opportunity to spread the disease due to the children younger than 10 years had less awareness and caution about the disease which consistent with the study of Dunais B et al[21] who found that the child reared by the caretaker

who responsible to take care less than 3 children had lower rate of carrier stage of *H. influenzae* than the child who reared by the caretaker with more than 3 children to care for.

The susceptibility of *H. influenzae* to antibio tics found 31.5% resisted to ampicillin which close to the study of Olsen SJ et al[16] who found 27% resistant and the study of Hashida K et al[14] which found 37.2% resistant. For the resistant *H. influenzae* to cotrimoxazole, chloramphenicol, and cefotaxime were 68.5%, 6.5%, and 1.1% respectively which were congruent with the study of Olsen SJ et al[16] which were 57%, 15% and 0% respectively. Antibiotic susceptibility test for *H. influenzae* isolated from the healthy child may have the clinical benefit by predicting the trend of antimicrobial susceptibility of the bacteria isolated from the patient. By many studies found the drug resistant of *H. influenzae* clinically isolated could be predicted by the results of drug susceptibility test of the bacteria isolated from the throat which could be useful for the consideration of antimicrobial usage for the patient. For antimicrobial susceptibility test, it is recommended to confirm the result with other methods such as agar dilution, broth microdilution, E-test which is one of the reliable method for antimicrobial susceptibility testing[22].

The family and involved organizations should be responsible to take care the pregnant women to prevent preterm labor, low birth weight baby, take care the child health especially those under 3 years old, the children in extended family with more than 5 members, and in the family with the children aged below 10 years due to these children have higher chance of getting the disease including the appropriate antibiotic usage when they had throat infection.

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ปัจจัยที่มีความสัมพันธ์กับการเป็นพาหะของเชื้อ *Haemophilus influenzae* และการดื้อยาต้านจุลชีพในเด็กสุขภาพดีของศูนย์เด็กเล็กในโรงพยาบาลส่งเสริมสุขภาพแห่งหนึ่ง

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บทคัดย่อ

การวิจัยนี้เป็นการศึกษาแบบภาคตัดขวางในเด็กที่มารับบริการที่ศูนย์เด็กเล็กของโรงพยาบาลส่งเสริมสุขภาพแห่งหนึ่ง เพื่อประเมินภาวะการเป็นพาหะและปัจจัยที่มีความสัมพันธ์กับการเป็นพาหะของ *Haemophilus influenzae* ในลำคอ รวมทั้งหาการดื้อต่อยาต้านจุลชีพของเชื้อ *Haemophilus influenzae* กลุ่มตัวอย่างเป็นเด็กอายุ 1½ - 5 ปี จำนวน 189 คน เป็นชาย 95 คน หญิง 94 คน เก็บข้อมูลโดยใช้แบบสอบถามผู้ปกครองชนิดตอบด้วยตนเอง ข้อมูลทุติยภูมิจากแฟ้มประวัติของศูนย์เด็กเล็ก การตรวจร่างกายและเก็บสิ่งส่งตรวจจากในลำคอ ระหว่างเดือนกรกฎาคม 2550 ถึง พฤศจิกายน 2550 วิเคราะห์ข้อมูลโดยใช้ ความถี่ ร้อยละ Chi-square test และ Multiple logistic regression ผลการศึกษาพบว่าร้อยละ 44.4 ของเด็กเป็นพาหะของ *H. influenzae* และในจำนวนนี้ พบว่าเป็นพาหะของ *H. influenzae* type b (Hib) ร้อยละ 1.1 ปัจจัยที่สัมพันธ์กับการเป็นพาหะของเชื้อ *H. influenzae* อย่างมีนัยสำคัญทางสถิติได้แก่ เด็กที่อายุน้อยกว่า 3 ปี ประวัติการคลอดก่อนกำหนด น้ำหนักแรกเกิดที่ต่ำกว่า 2,500 กรัม การมีเด็กอายุน้อยกว่า 10 ปีมากกว่า 2 คนในบ้าน การมีสมาชิกในบ้านมากกว่า 5 คน มารดามีอาชีพรับราชการหรือรับจ้าง เด็กอยู่ในครอบครัวขยาย และการไม่ได้รับวัคซีนป้องกันเชื้อฮิบ Hib ปัจจัยที่มีอิทธิพลต่อการเป็นพาหะของเชื้อ *H. influenzae* ได้แก่ ประวัติการคลอดก่อนกำหนด และ การมีเด็กอายุน้อยกว่า 10 ปี มากกว่า 2 คนในบ้าน จากการศึกษาความไวของเชื้อต่อยาต้านจุลชีพพบว่า *H. influenzae* ร้อยละ 68.5 ดื้อต่อยา cotrimoxazole ร้อยละ 31.5 ดื้อต่อยา ampicillin และร้อยละ 1.1 ดื้อต่อยา cefotaxime ดังนั้นครอบครัวและหน่วยงานที่เกี่ยวข้องกับการดูแลเด็กควรมีการวางแผนการป้องกันการแพร่เชื้อและลดโอกาสการเป็นพาหะของเชื้อ *H. influenzae* ตั้งแต่การตั้งครรภ์ โดยการป้องกันการคลอดก่อนกำหนด ทารกแรกเกิดน้ำหนักน้อย การดูแลสุขอนามัยในครอบครัวที่มีจำนวนสมาชิกหลายคน โดยเฉพาะอย่างยิ่งในครอบครัวที่มีเด็กอายุน้อยกว่า 10 ปีในบ้าน ตลอดจนการฉีดวัคซีนป้องกันโรคที่เหมาะกับความเสี่ยงของแต่ละครอบครัว เพื่อป้องกันการแพร่ของเชื้อ รวมถึงการใช้ยาต้านจุลชีพที่เหมาะสม

คำสำคัญ: การเป็นพาหะในลำคอ เชื้อ *H. influenzae* เด็กสุขภาพดี ศูนย์เด็กเล็ก การดื้อยาต้านจุลชีพ

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