

Prevalence of variant bacteria in oropharyngeal colonization of Iranian children

Alireza Fahimzad¹, Benyamin Karimi¹, Mohammad Ali Malekan¹, Ahmad Reza Shamshiri², Masoomeh Mohkam¹, Mostafa Sharifian¹, Saeed Maham¹

1. Pediatric Infectious Research Center, Shaheed Beheshti Medical University, Tehran, Iran

2. School of Health and Institute of Health Research, Department of Epidemiology and Biostatistics, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: On the base of relation between oropharyngeal colonization of bacterial pathogens and etiology of invasive and noninvasive pediatric diseases, we evaluated their prevalence and relationship between them.

Materials and methods: The colonized bacteria in oropharynx of 296 children between 2-6 years old from 7 day care centers of Tehran were isolated and determined by specific diagnostic tests.

Results: Prevalence rates of bacterial colonization include *Streptococcus pneumoniae* (32.4 %), *Haemophilus influenzae* (23.9%), *Moraxella catarrhalis* (13.5%), *Neisseria meningitidis* (12.1%) and *Pseudomonas aeruginosa* (2.7%). Our study showed the reverse relationship between colonization rate of *Streptococcus Pneumoniae* and *Haemophilus influenzae* and between *Neisseria meningitidis* and *Haemophilus influenzae*.

Conclusion: Determination of colonized bacteria in oropharynx of healthy children and relationship between them can be helpful to find the ways to interfere with their colonization and prevention of diseases due to them.

Keywords: *Oropharyngeal colonization, Bacterial colonization, Day care center.*

(*Iranian Journal of Clinical Infectious Diseases 2008;3(1):25-28*).

INTRODUCTION

The oropharynx of children is colonized by extended spectrum of invasive and noninvasive microorganisms such as: *Haemophilus influenzae*, *Neisseria meningitidis* and corynebacteria species (spp.). Microbial colonization on mucosal surface is a dynamic process, so it could gain or lose the bacteria and obtain them at other times (1). Defecting factors of specific and nonspecific

immunity including lysozyme production, local inhibitory antibodies and microbial status in oropharynx are effective in developing of colonized microbial flora (2).

Thus disturbance of colonized microbial flora and its balance by different agents can result in pathogenesis of some of these microorganisms. The identified risk factors that increase probability of pathogenic microorganisms colonization include: living in crowded areas such as day care centers, repeated respiratory infections and increased consumption of antibiotics (3).

Received: 9 April 2007 Accepted: 30 September 2007

Reprint or Correspondence: Alireza Fahimzad, MD, Pediatric Infectious Research Center, Mofid Children's Hospital, Shariati St., Tehran, Iran.

E-mail: safahimzad@yahoo.com

26 Variant bacteria in oropharyngeal colonization

Inappropriate antibiotic usage replaces highly resistant microorganisms instead of susceptible ones (3, 4). So healthy children can be important resource of some antibiotic resistant microorganisms and spreading of these microbial agents to other children and their family members (1).

This study was performed to find the colonization rate of above mentioned microorganisms in children oropharynx and to evaluate the relationship between them, in day care centers of Tehran.

PATIENTS and METHODS

Specimens were collected from oropharynx of 296 children of 7 day care centers between 2-6 years old in Tehran during 3 months in winter 2006. The day care centers were selected by cluster sampling.

After obtaining written informed parental consent, microbiologist took Specimens from posterior area of tonsils and pharynx and tonsils crypts aseptically with sterile swab. They were cultured immediately next to flame on chocolate agar media. All the cultured media were transferred to research center and incubated in 37 °C for 24-48 hrs in anaerobic Jars by candle to produce CO₂. The primary diagnosis of bacteria was performed by gram staining of smears from colonized bacteria and their colony morphology. The confirmatory procedures were performed for diagnosis of genus and species of isolation such as Catalase, oxidase, requirement to X and V factors, ALA (δ - Amino Levulinic Acid) test, optochin and bacitracin susceptibility, type of hemolysis, growth on specific media such as Thayer- martin agar and bile esculin agar. Relation of colonized bacteria with age and sex of children, parental education level, smoking exposure, history of respiratory diseases or antibiotic consumption during recent month, residence and staying time in day care centers and

number of siblings under 5 years old was evaluated.

Simple random cluster sampling method was used to select study day care centers in Tehran. Data were analyzed by logistic regression for survey data methods within STATA/SE 8.0 to determine the relationship and co-existence of bacteria in oropharynx of children.

RESULTS

Identified microorganisms from oropharynx of 296 day care centers children are shown in table- 1.

Table 1. Oropharyngeal colonized bacteria from children of day care centers in Tehran

Isolated bacteria	Number	Percentage
Streptococcus pneumoniae	96	32.4%
Haemophilus influenzae	71	23.9%
Neisseria species	132	44.5%
Neisseria meningitides	36	12.1%
Moraxella catarrhalis	40	13.5%
Staphylococci	48	16.2%
Corynebacteria	40	13.5%
Pseudomonas aeruginosa	8	2.7%

The relationship between isolated microorganisms is shown in table- 2.

Table 2. Relationship between isolated microorganisms from children of day care centers in Tehran

Two compared microorganisms	Odd ratio	P
S. pneumoniae & H. influenzae	0.14	<0.001
N. meningitides & H. influenzae	0.31	<0.001
N. meningitides & M. catarrhalis	1.03	0.04
N. meningitides & P. aeruginosa	1.06	0.04
Corynebacterium & Staphylococci	1.06	<0.001

There was no significant association between isolated bacteria and determined factors (such as age, sex, parental educational level) in children except relationship between Haemophilus influenza colonization and respiratory infection history during recent month (P =0.002).

DISCUSSION

Our study showed oropharyngeal microbial colonization prevalence in Iranian children and possible relationship between them. On the base of present evidences, this study is the first Iranian research in pediatric field. Some recent studies revealed that microflora of mouth, oropharynx and nasopharynx are different. Moreover oropharynx has more variation of microbial colonization compared with other parts (5,6,7).

Colonization of three common microbial agents of respiratory infections including *S.pneumoniae*, *H. influenzae* and *M. catarrhalis* in this study are comparable with similar studies. In one study 156 healthy children between 1 month to 5 years old in 2 Japanese day-care centers were evaluated. Microbial colonization prevalence for each of these three bacteria was 60.3%, 53.2% and 34.2% respectively (8). Colonization prevalence rates of above bacteria in 586 children between 2 month to 2 years old of Portugal day care centers were 47%, 72% and 54% respectively (9).

The lower age of children in two referred studies, vaccination history for *H. influenzae* in those countries and inappropriate antibiotic consumption in Iran might be the possible explanations for low prevalence rate of mentioned bacteria in our study.

The reverse relationship between *Strep. pneumoniae* and *H. influenzae* colonization in our study may result from common cellular receptors such as superficial sialic acid, fibronectin and platelet activator factor (PAF) receptors, common IgA protease enzyme and some common superficial protein ligands that compete with each other (10,11,12).

It was reported in a review article in 2004 that prevalence rate of *N. meningitidis* colonization in healthy children below 4 years old was 3% reaching to 24-37% in 15-24 years old (13). In our study this colonization rate was variable between 2-30% in different day care centers. (average=

12.1%). An interesting point was reverse relationship between colonization rate of *N. meningitidis* and *H. influenzae*. They have common receptors and need binded iron of human transferrin. Both of them can induce biofilm layer for outer epithelial cells, so they compete with each other in epithelial adhesion (14,15,16,17).

Pseudomonas aeruginosa oropharyngeal colonization in children is developed transiently following ingestion of fresh fruits and vegetables.

This colonization increases in hospital environment due to antibiotic consumption and alteration in oral microbial flora (18). *P. aeruginosa* colonization prevalence in this study was 2.7% comparable with other studies (19).

Since there is a significant association between bacterial prevalence in children oropharynx and prevalence rates of invasive diseases, determination of colonization rate and rational relation between them can be helpful to find the ways to interfere with their colonization and prevention of diseases due to them.

ACKNOWLEDGMENT

We are pleased from the supports of Iran National Science foundation (INSF) and cooperation of Pediatric Infectious Research center (PIRC) personnel at Mofid children hospital in this study and sincerely appreciate them.

REFERENCES

1. Sulikowska A, Grzesiowski P, Sadowy E, Fielt J, Hryniewicz W. Characteristics of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* isolated from the nasopharynxes of asymptomatic children and molecular analysis of *S. pneumoniae* and *H. influenzae* strain replacement in the nasopharynx. *J Clin Microbiol* 2004;42(9):3942-49.
2. Fox JP, Cooney MK, Hall CE, Foy HM. Influenzavirus infections in Seattle families, 1975-1979. II. Pattern of infection in invaded households and relation of age and prior antibody to occurrence of infection and related illness. *Am J Epidemiol* 1982;116(2):228-42.

28 Variant bacteria in oropharyngeal colonization

3. Garcia-Redriguse JA, Fresnadillo-Martines MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother* 2002;50:59–73
4. Pradier C, Dunais B, Largillier R, Carsenti-Etesse H, Bernard E, Scheimberg A, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* in children's day-care centers: 10-month follow-up study in Nice, France. *Clin Microbiol Infect* 1997;3(6):705-708.
5. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* 2005;43(11):5721-32.
6. Lieberman D, Shleyfer E, Castel H, Terry A, Harman-Boehm I, Delgado J, et al. Nasopharyngeal versus oropharyngeal sampling for isolation of potential respiratory pathogens in adults. *J Clin Microbiol* 2006;44(2):525-28.
7. Feigin RD, Cherry JD, Demmler GJ, Kaplan SL. *Textbook of pediatric infectious Diseases*. 5th edition. New York: W.B. Saunders; 2004: 107-14.
8. Masuda K, Masuda R, Nishi JI, Tokuda K, Yashinaga M, Miyata K. Incidences of nasopharyngeal colonization of respiratory bacterial pathogens in Japanese children attending day-care centers. *Pediatr Int* 2002;44:376–80.
9. Principi N, Marchisio P, Schito GC, Mannelli S. Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. *Ascanius project collaborative Group. Pediatr Infect Dis J* 1999;18:517–23.
10. Vimr E, Lichtensteiger C, Steenbergen S. Sialic acid metabolism's dual function in *Haemophilus influenzae*. *Mol Microbiol* 2000;36(5):1113-23.
11. Avadhanula V, Rodriguez CA, Ulett GC, Bakaletz LO, Adderson EE. Nontypeable *Haemophilus influenzae* adheres to intercellular adhesion molecule 1 (ICAM-1) on respiratory epithelial cells and upregulates ICAM-1 expression. *Infect Immun* 2006;74(2):830-38.
12. Plaut AG. The IgA1 proteases of pathogenic bacteria. *Annu Rev Microbiol* 1983;37:603-22.
13. Siamak P, Yazdankhah W, Dominique A. *Neisseria meningitidis*: an overview of the carriage state. *J Med Microbiol* 2004;53:821-32.
14. Muenzner P, Naumann M, Meyer TF, Gray-Owen SD. Pathogenic *Neisseria* trigger expression of their carcinoembryonic antigen-related cellular adhesion molecule 1 (CEACAM1; previously CD66a) receptor on primary endothelial cells by activating the immediate early response transcription factor, nuclear factor- κ B. *J Biol Chem* 2001;276(26):24331-40.
15. Clarke TE, Tari LW, Vogel HJ. Structural biology of bacterial iron uptake systems. *Curr Top Med Chem* 2001;1(1):7-30.
16. Jarosik GP, Maciver I, Hansen EJ. Utilization of transferrin-bound iron by *Haemophilus influenzae* requires an intact tonB gene. *Infect Immun* 1995;63(2):710–13.
17. Tsai J, Dyer DW, Sparling PF. Loss of transferrin receptor activity in *Neisseria meningitidis* correlates with inability to use transferrin as an iron source. *Infect Immun* 1988;56(12):3132–38.
18. Remington JS, Schimpff SC. Occasional notes. Please don't eat the salads. *N Engl J Med* 1981;304(7):433-35.
19. Döring G, Hörz M, Ortel J, Grupp H, Wolz C. Molecular epidemiology of *Pseudomonas aeruginosa* in an intensive care unit. *Epidemiol Infect* 1993;110(3):427-36.