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RESEARCH PAPER

Prevalence of nasopharyngeal carriage of *Streptococcus pneumoniae* in children 7 to 14 years in 2016: A survey before pneumococcal conjugate vaccine introduction in Iran

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ABSTRACT

Streptococcus pneumoniae is a common cause of community-acquired pneumonia, meningitis, and otitis media in children. The aim of this study was to determine the prevalence of nasopharyngeal carriage of *Streptococcus pneumoniae* among children in the city of Hamadan, west of Iran. In this cross-sectional study, 532 students aged 7 to 14 years old from Hamadan were enrolled during the period from February to April 2016. Children were recruited using multi-stage sampling method. Informed consent form was obtained from parents of children. A researcher developed checklist was completed for every child by interviewer and samples of the throat of children were taken by swap method from the nasopharyngeal area. Descriptive statistics and chi square test were used to describe the study population. This study was approved by the Committee on Ethics of Hamadan University of Medical Sciences (IR.UMSHA.REC.1394.66). Prevalence of nasopharyngeal carriage of *S. pneumoniae* in children was 12.03% (95%CI: 9.38–15.10). About 37% (196 persons) of study population were male and 63% were female. Sixty four percent (345 people) of the studied population were from district two in Hamadan and others from District one. Prevalence of nasopharyngeal carriage of *S. pneumoniae* by sex was 13.77% (95% CI: 9.27–19.40) in males and 11.02 % (95% CI: 7.87–14.85) in females (P = 0.345). Considering the high prevalence of nasopharyngeal carriage of *Streptococcus pneumoniae* in children studied in Hamadan, pneumococcal conjugate vaccine (PCV) is recommended to be integrated into the Iran's National Immunization Program.

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Introduction

Streptococcus pneumoniae (*S. pneumoniae*) is a gram-positive bacterium that is one of the most important causes of invasive pneumococcal diseases including pneumoniae, septicemia and bacterial meningitis among children.[1], [2] The most common manifestation of severe pneumococcal infection is pneumonia.[3] Invasive pneumococcal disease occurs when pneumococcus from the respiratory tract enters the bloodstream or cerebrospinal fluid and causes meningitis, bacteremia and sepsis.[2]

Pneumococcus is often located in the upper respiratory tract. Human nasopharynx is only natural reservoir for it. Pneumococcus is transmitted through contact with respiratory droplets. Nasopharyngeal carriage is the first stage for pathogenesis of pneumococcal infection.[4,5] This bacterium is a major cause of death in the world.[3]

In 2013, around 935,000 deaths of children under 5 years old were due to pneumonia, of which, approximately 15% were due to *S. pneumonia* [6] *Pneumococcal conjugate vaccines (PCV10/PCV13) are available for S. pneumoniae,* which covers 10 and 13 common serotypes, respectively. These vaccines reduce the severity of pneumococcal disease and carriers of vaccine serotypes in human.[7,8]

World Health Organization recommends that vaccine of pneumococcus should planned in the country's vaccination program, especially in countries with high child mortality rates (mortality rate for children under five year, more than 5 death per 1000 birth).[9]

Nasopharyngeal carrier state of *S. pneumoniae* mainly occurs in the first year of life. Prevalence of nasopharyngeal carriage of pneumococcus in children under 2 years varies from 30% up to 62%[10] and in children under 5 years varies from 40% to over 90%.[4,5]

Results of a meta-analysis in Iran declare that prevalence of nasopharyngeal carrier state of *S. pneumoniae* is 18% (95%CI: 14%, 23%).[11] Moreover, it is necessary to address the nasopharyngeal carrier state of *S. pneumoniae* in Iran before introduction of PCV vaccine. This study provides evidence to

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better decision on PCV introduction and epidemiological profile of *S. pneumoniae*.

In a study conducted in Tehran on children less than 10 years, nasopharyngeal carriage of *S. pneumoniae* was 44.1%.[12] Diagnosis of bacteria is usually based on the observation of *S. pneumoniae* in the specimen of sputum or bacterial growth in the culture of sputum, pleural fluid, blood and other respiratory specimens.[13,14]

Prevalence of nasopharyngeal carriage among population and young children is epidemiologically important. The reason for this epidemiological role of carriage of *S. pneumoniae* is the risk of circulation of pneumococcus-following colonization. Population density is an effective factor in increasing the colonization of bacteria. Due to the transfer of bacteria from carriers to other people, especially in closed societies and environments, use of vaccination and eradication of bacteria in carriers is important.[10] This study was conducted to determine the prevalence of nasopharyngeal carriage of *S.pneumoniae* and its related factors in children aged 7–14 years in Hamadan, west of Iran, during 2016.

Results

Overall, 532 healthy children were enrolled for study. Baseline characteristics of the study population are shown in Table 1. The results of the study showed that 36.84% of students (196 people) were male and 63.16% (336 people) were female. The mean and standard deviation of the students' age (Mean± SD) were 10.83 ± 2.35, household size was 3.94 ± 0.96, and the number of rooms was 1.85 ± 1.99, respectively. Majority of educational level mothers and fathers were elementary 42.85% (263 person) and 45.8955% (244 person), respectively (Table 1). The prevalence of nasopharyngeal carriage of *S. pneumoniae* among students was 12.03% (95% CI: 9.38, 19.4). The corresponding value among male students was 13.78% (95% CI%: 9.27, 15.10) and 11.01% (95% CI%: 7.87, 14.85) in females (P = 0.345).

The prevalence of nasopharyngeal carriage in the age group of 10 years and younger was 12.75% (CI%: 8.88–17.51) and in the age group of 10 years and older 11.39% (CI%: 7/92–15/69), but was not statistically significant (P = 0.630). The prevalence of nasopharyngeal carriage in male in the age group of 10 years and younger was 19.78% (CI%: 12.16–29.44) and in the age group of 10 years and older 8.57% (CI%: 3.99–15.64). This relationship was statistically significant (P = 0.023). Also, the prevalence of nasopharyngeal carriage in female in the age group of 10 years and younger was 8.75% (CI%: 4.86–12.24) and in the age group of 10 years and older 13.07% (CI%: 8.46–18.96). However, the relationship was not statistically significant (P = 0.207).

The prevalence of nasopharyngeal carriage in students who have smoker parents was 18.03%. The corresponding value for other students was 8.88% (P = 0.002). Findings from multivariate logistic regression analysis showed that smoking was statistically significant determinant of nasopharyngeal carriage prevalence among study participants (OR = 1.85). Details on the magnitude of odds ratios (OR) of included variables in the model has been shown in Table 2.

Table 2. Summary results of multivariate logistic regression model on determinants of nasopharyngeal carriage prevalence.

Variable	B	S.E.	P Value*	Odds Ratio (95% Confidence Intervals)
Region	0.052	0.354	0.833	1.05 (0.52– 2.10)
Sex	0.292	0.336	0.385	1.33 (0.69– 2.58)
Age	0.067	0.057	0.239	1.07 (0.95– 1.19)
Household	0.063	0.140	0.652	1.06 (0.80– 1.40)
Room	−0.037	0.119	0.759	0.96 (0.76–1.21)
Sleeping	−0.073	0.304	0.810	0.93 (0.51– 1.68)
Smoking	0.619	0.271	0.022	1.85 (1.09– 3.15)

*Adapted from multivariate logistic regression model

Table 1. Nasopharyngeal carriage of *Streptococcus pneumoniae* by baseline characteristics of students.

		Nasopharyngeal Carriage N (%)		
Variable	Levels of Variable	Positive	Negative	P Value
Educational Level Mother	Illiterate	4(8.51)	43(91.49)	0.551
	Elementary School	35(13.31)	228(86.69)	
	Secondary School	22(12.36)	156(87.64)	
	High School	3(6.82)	41(93.18)	
Educational Level Father	Illiterate	7(15.22)	39(84.78)	0.172
	Elementary school	36(14.75)	208(85.25)	
	Secondary school	16(9.52)	152(90.48)	
	High School	5(6.86)	68(93.15)	
Gender	Male	27(13.78)	169(86.22)	0.345
	Female	37(11.01)	299(88.99)	
Children's sleep mode	With Parents	18(11.92)	133(88.08)	0.961
	Without Parents	46(12.7)	335(87.93)	
Status of Parent Smoking	In Home	33(18.03)	150(81.97)	0.002
	Outdoor	31(8.88)	318(91.12)	
Age Group Total	≤ 10 year old	32(12.75)	219(87.25)	0.630
	> 10 year old	32(11.39)	249(88.61)	
Age Group for Male	≤ 10 year old	18(19.78)	73(80.22)	0.023
	> 10 year old	9(8.57)	96(91.43)	
Age Group for Female	≤ 10 year old	14(8.75)	146(91.25)	0.207
	> 10 year old	23(13.07)	153(86.93)	

Discussion

The results of this study showed the prevalence of nasopharyngeal carriage of *S.pneumoniae* in healthy students was 13.77% (CI: 9.27–19.4). In various studies conducted in Iran, the prevalence of nasopharyngeal carriage of this bacterium and its serotypes has been reported differently. In the studies, the prevalence of the nasopharyngeal carriage has been reported from 5.9% to 44.1%. Similar studies include *Mirzaei et al.*,[15] *Bokaeian et al.*,[16] *Senaei Dashti et al.*,[12] the prevalence of nasopharyngeal carriage of *S. pneumoniae* was 13.9%, 15.7% and 44.1%, respectively.

According to the last meta-analytical study conducted by *Hosseini et al* [11]in 2015 in Iran, the prevalence of nasopharyngeal carriage of this bacterial in children under the seven years and in children upper seven age was 18% and 13%, respectively,the results were consistent with our study. In the study of *Zhou JY et al.*,[17] the prevalence of nasopharyngeal carriage of *S. pneumoniae* in children aged 6 years and below was 34% (confidence intervals 26– 44%), which was more two times than the results of our study. It seems the main reason for inconsistency of reporting the prevalence of nasopharyngeal carriage of *S. pneumoniae* are epidemiological profile of studied subgroups, time and season of sampling. We did not observe any association between nasopharyngeal carriage of *S. pneumoniae* and age, whereas some studies have reported that the age is a risk factor.[11,18] Prevalence of nasopharyngeal carriage in children younger than 2 years, 2 to 6 years old and 7 years and above was 37%, 32% and 7.2%, respectively. Results showed that with increasing age, the prevalence of bacterial colonization is lower. In our study, in the lower age group, the prevalence of bacterial colonization was higher. In contrast, in the age group over 10 years, the prevalence of bacterial colonization was lower.

The prevalence of the nasopharyngeal carriage of *S. pneumoniae* is different according to geographical areas, age groups, economic conditions, and seasonal variation. Population density is also an effective factor in increasing bacterial colonization. Due to the transmission of bacteria from carriers to other people, especially in closed communities and environments, the use of vaccination and eradication of bacteria in carriers is very important.

The prevalence of the nasopharyngeal carriage of *S. pneumoniae* in students whose parents are smoking at home is higher than those who do not smoke at home. This seems to be logical because smoking and exposure to cigarette smoke increase the chance of developing respiratory diseases. Therefore, it can interfere with the prevalence of colonization. Findings from running logistic regression model were in comply with univariate analysis, as well.

The level of education parents can be effective in developing the colonization this bacteria in nasopharyngeal in the children, so whatever higher the level of parental education, the probability of occurrence the colonization this bacteria in nasopharyngeal is lower, which is the result of our study. This suggests that parents with a higher level of education are more likely to be aware of the disease and the means of transmission. Also, at the time of illness, the children are more likely to go to the doctor on a timely basis and treat it before becoming pregnant and chronically ill.

According to studies conducted in this subject, it is expected that after the integration of the pneumococcal vaccine into the national vaccination program, the epidemiological characteristics of the bacterium will change in the countries, and the prevalence of nasopharyngeal carriage of the bacterium will be decreasing. According to the guidance of World Health Organization, it is necessary to integrate the pneumococcal vaccination program into the national immunization program of all countries (especially in countries with high mortality rates in children).[6,11,17–19]

This study has some limitations. We did not identified serotypes of *S. pneumoniae*. Moreover, antibiotic resistances of the bacteria were not investigated. In case of interpretation of study results, it should be noted that different microbiological tests were used in the different published papers. Despite these limitations, to our knowledge, there is no published data regarding the prevalence nasopharyngeal carriage of *S. pneumoniae* in Hamadan city. Our findings might be useful for policy makers and public health authorities as well-informed evidence to decision on PCV introduction.

Conclusions

A high prevalence of nasopharyngeal carriage of *S. pneumoniae* in healthy students was observed. Moreover, findings revealed that smoking by parents of children is potential determinant of nasopharyngeal carriage prevalence among study participants. Accordingly, it is highly recommended to introduce pneumococcal vaccines in Iran and integrate the appropriate vaccine into Iran's routine immunization program.

Methods

Study population

This cross- sectional survey was conducted among healthy children aged 7 up to 14 years from February to April 2016 in Hamadan. Hamadan Province is located in the west of Iran and Hamadan city is the center of this province (Figure 1). The province had a population of 1,758,268 people with marginal located slum areas (https://www.amar.org.ir/english).

Students from both districts of Hamadan city were recruited by multistage sampling method. We have selected targeted schools from each district by random sampling and finally recruited 532 eligible students by systematic random sampling and proportion to size approach. Nobody of study participants received PCV vaccination while enrolled to the study.

Data collection

After obtaining written informed consent parents/guardians by educated interviewers, we completed a researcher- developed information sheet to obtain minimum data on baseline characteristics of students include sex, age, status of residency and levels of parent's education. People complete their elementary school at age 12 years, secondary school at 15 years and high school at 18 years. Parents of children were asked by interviewers to complete the information sheet along with informed consent form if they were agree.

Figure 1. Geographical location of Hamadan province in west of Iran.

Sampling and bacteriologic methods

After obtaining written informed consent forms using a sterile swab, samples were provided from the nasopharyngeal area of students. The samples with the transportation media were transferred to the microbiology laboratory of the medical school and cultured immediately for *S. pneumoniae*. The nasopharyngeal samples were cultured on enriched chocolate agar plates and selective sheep blood agar plates containing 5 mg/mL gentamicin. The plates were incubated in 5% CO2 at 37oC, overnight. *S. pneumoniae* was identified by colony morphology, ∝-hemolysis, optochin sensitivity, and bile solubility. Detail has been described elsewhere.[20,21]

Data analysis

Descriptive statistics including mean and standard deviation, frequency, percentage and tables were used to describe the studied population. According to the study objectives, prevalence of nasopharyngeal carriage *S. pneumoniae* was reported with 95% confidence interval. The chi-square test was used to determine the relationship between the prevalence of nasopharyngeal carriage *S. pneumoniae* and qualitative variables. We have run a multivariate logistic regression model to address the determinants of nasopharyngeal carriage prevalence among study participants. Results of logistic regression has been reported as OR along with its 95% CIs. Stata software version 11.2 was used for statistical analysis. Confidence intervals around the point estimates of prevalence were reported. P-Values less than 0.05 were considered statistically significant.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Declarations

Ethics approval and consent to participate

This study was approved by the Committee on Ethics of Hamadan University of Medical Sciences (IR.UMSHA.REC.1394.66). We have obtained written informed consent forms from parents/guardians by educated interviewers to participate in the study.

Availability of data and material

The datasets used and analyses during the current study are available from the corresponding author on reasonable request.

Authors' Contribution

All authors have approved the manuscript. MK has established first idea data analysis and drafted manuscript, SMH, MYA, SHH and HE helped to design and conduct the study. All authors have had substantial contribution in data gathering, Sampling, manuscript drafting, and critical revision of manuscript and data analysis.

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