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Nasopharyngeal carrıage rates of streptococcus pneumonıae and changes after conjugate pneumococcal vaccine.

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Abstract

***Background: Streptococcus pneumoniae* infection in children usually begins with nasopharyngeal colonization that may be a significant risk factor for invasive pneumococcal disease. In this study it is aimed to determine the nasopharyngeal pneumococcal carriage rate in preschool-aged children, penicillin resistance rates of the isolated strains, risk factors for *S. pneumoniae* carriage.**

***Materials and methods:* Two hundred and fourteen children attending preschool were included in our study. The demographic characteristics and possible risk factors for pneumococcal carriage were investigated. Chi-square test and logistic regression model were used for statistical analysis.**

***Results:* Nasopharyngeal samples were taken from 214 healthy preschool children. No statistical difference was found between carrier and non-carriers with respect to age, gender, having respiratory tract infection within the last three months, presence of smokers in the family and number of people in the family. Thirty four of 214 children were unvaccinated against pneumococcus and 6 patients (17.6%) of these 34 children were found as *S. pneumonia* carrier. While 50% of pneumococcal isolates were susceptible to penicillin, the rate of high-level resistance to penicillin was 16.7% and intermediate level resistance rate was 33.3%. The pneumococcal carriage was found significantly increased in unvaccinated children compared to vaccinated children ($P<0.0001$).**

***Conclusion:* In our study the nasopharyngeal pneumococcal carriage rate was 2.8% in children aged between 4 and 6 years. This rate is lower than the data previously reported. Carriage was not seen in vaccinated children in our study. We think that routine pneumococcal vaccination is the major factor underlying this decrease. These results should be supported with multicenter studies.**

Keywords: Pneumococcal infections, Streptococcal infections, Vaccination.

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Background

Streptococcus pneumoniae is the most frequent cause of community-acquired bacterial pneumonia, bacteraemia, acute sinusitis, and acute otitis media. The source of the pneumococcal infection that may cause significant morbidity and mortality is mainly the asymptomatic carriers [1]. Pneumococcal infection usually begins with the nasopharyngeal colonization, and nasopharyngeal colonization is an important risk factor for development of invasive disease. Nasopharyngeal colonization by *S. pneumoniae* usually begins within the first two years of life depending on the epidemiological and socio-economic factors. The incidence of acute otitis media is higher in young children with colonization than children without colonization. The carriage rate varies according to age, geographic region and the population [1, 2]. Carrier people transmit the bacteria most commonly through respiratory tract. According to the local studies conducted

in our country, nasopharyngeal carriage rate was reported between 13% and 43% [3-6]. However there is no reported epidemiological study after the routine vaccination program against pneumococci began in 2008.

10 million children around the world die every year, and one-fifth of deaths are due to pneumonia. The agent in about half of the severe pneumonia cases is *S. pneumoniae* [7]. According to the data from United States of America, 13 thousand bacteraemia, 700 meningitis, 5 million acute otitis media cases are seen each year caused by *S. pneumoniae* and because of these infections a large number of deaths have been reported [1].

Until recent years these infections are used to be easily treated with penicillin but after 1960 treatment failures began to appear along with the reports of penicillin-resistant strains from various parts of the world. In the following years, the rates of penicillin resistance gradually increased and multi-drug resistant strains arise. The presence of penicillin and multi drug resistant strains in healthy carriers makes empirical treatment difficult [5, 8]. The resistance patterns show great differences among regions but also changes may occur over time in the same area. When the penicillin resistance in pneumococcus isolated from different groups in different regions were examined, the results were observed to be quite different from each other.

In this study we aimed to determine nasopharyngeal pneumococcal carriage rate, penicillin resistance of these strains in healthy preschool children in our community, evaluate the risk factors for carriage and the impact of 7 valent conjugate pneumococcal vaccinations that has been in routine vaccination program since 2008 in our country.

Material and Methods

This study was conducted in the province of Ordu between October and December 2012. Nasopharyngeal swabs of 214 healthy preschool children aged between 4-6 years of age were obtained. Necessary permissions from the provincial education directorates have been obtained. Written approval was taken from parents of the children participating in the study. The children of the parents who disapprove the study were excluded. A questionnaire including demographic characteristics, history of upper respiratory tract infection within the last three months, pneumococcal vaccination status, number of family members, and the presence of smoker in the family was filled out by the parents. The data were statistically analysed in terms of carriage and penicillin resistance.

Nasopharyngeal swab samples were taken with sterile dacron-tipped swab rods by the same researcher. The taken samples delivered to Microbiology Laboratory of the Ordu University Faculty of Medicine Training and Research Hospital, in the transport medium. Samples were inoculated onto 5% sheep blood agar and were incubated at 37°C in the oven with 5-10% CO_2 for 24-48 hours. Following incubation, *S. pneumoniae* isolates were identified by standard microbiological procedures; alpha-haemolysis, Gram stain characteristics, catalase activity, Optocin sensitivity (5 µg Bioanalyse OP) and bile solubilities were evaluated [9, 10]. Then, oxacillin disk (1 mg) (Bioanalyse) was used for determination of penicillin resistance. Pneumococcus which creates ≥ 20 mm zone around the disc was considered penicillin-susceptible, while ≤ 19 mm zone was resistant. Resistance to penicillin in oxacillin-resistant strains has been verified by E-test (Liofilchem, Italy) method. Penicillin susceptibility tests were interpreted according to the criteria set by Clinical Laboratory Standardts Institute (CLSI). *S. pneumoniae* ATCC 49619 strains was used as control strain.

Statistical Analysis

The association of the prevalence of pneumococcal carriage with potential risk factors for streptococcal infection was analysed using logistic regression models. The estimates of the association were expressed as odds ratios (OR) and 95% confidence intervals (CI). Chi-square test was used for comparison of variables. *P* value <0.05 was considered as statistically significant. All statistically analyses were performed SPSS version 16.0.

Results

Nasopharyngeal samples were taken from 214 (109 male and 105 female) healthy preschool children aged between 4 and 6. The mean age was 4.8 years. *S. pneumoniae* was isolated in 6 of 214 (2.8%) of nasopharyngeal swab samples. The distribution of *S. pneumoniae* carriage according to risk factors was shown in Table 1. Fifty percent of pneumococcal isolates were susceptible to penicillin. High-level resistance was detected in 16.7% and intermediate level resistance in 33.3%. All of the identified carriers were in the 34 unvaccinated group of children. The pneumococcal carriage was found significantly increased in unvaccinated children compared to vaccinated children (χ^2= 32.681, *P*<0.0001). No statistical difference was found between carrier and non-carriers with respect to age (χ^2=2.148, *P*=0.315), gender (χ^2=0.611, *P*=0.434), respiratory tract infection history within the last three months (χ^2=1.326, *P*=0.249), presence of smokers in the family (χ^2=0.137, *P*=0.711) and number of people in the family (χ^2=0.691, *P*=0.751). Although not statistically significant, male gender, increased number of people in the family, 4 and 6 years of age and not having recent upper respiratory tract infection were found to be associated with increased pneumococcal carriage.

Discussion

Pneumococcal nasopharyngeal carriage creates a significant risk factor for the occurrence of invasive disease and spread of resistant strains in the society. Although the first penicillin-resistant pneumococcus was isolated from an adult patient, the first isolated pneumococcus resistant to multiple antibiotics was found in children. In

Table 1: *Potential risk factors associated with nasopharyngeal carriage of S. pneumoniae in the logistic regression equation*

Variable	No.	Total No.	Prevalence (%)	OR	95% CI	*P*-value
Sex						
Male	4	109	3,7	1.962	0.352-10.945	0,442
Female	2	105	1,9	-	-	
Age (years)						
4	2	40	5.0			
5	2	124	1.6	0.311	0.042-2.287	0.251
6	2	50	4.0	0.792	0.107-5.883	0.819
Upper respiratory tract infection during last three months						
Yes	3	152	2.0	-	-	
No	3	62	4.8	2.525	0.496-12.870	0.265
Smokers in the family						
Yes	4	127	3.1	1.382	0.248-7.716	0.712
No	2	87	2.3	-	-	
Number of family members						
3	1	57	1.8	-	-	
4	3	108	2.8	1.600	0.163-15.741	0.687
5	2	49	4.1	2.383	0.209 -27.110	0.484
Vaccination status						
Yes	0	180	0			
No	6	34	17.64	-		<0.0001

OR: Odds Ratio; The final model fit was tested using Hosmer-Lemeshow test (p=0.666).

later studies it was determined that multi-resistant strains frequently caused infection in children.

The exact cause of this condition is unknown, probably, respiratory tract infections and so the use of antibiotics is considered to be more frequent in children [11]. In our country, the intermediate level resistance was reported as 0-51% and there were studies reporting high levels resistance to penicillin between 0-17% [12, 13, 14]. Looking at the world situation, resistance to penicillin has been reported in Spain as 40%, in South Africa as 50% and in Hungary up to 70% [15, 16, 17].

In the studies related to pneumococcal nasopharyngeal carriage and penicillin resistance in healthy children in our country; Gazi et al. [18] determined the carriage in children between 6-14 age as 23.4%, high-level resistance to penicillin as 7%, mid- level penicillin resistance as 17.9%; Ciftci et al. [5] reported the carriage in healthy children from 2 months to 12 years of age as 30%, the high and mid-level resistance to penicillin as 2.7% and 32.7% respectively. In the study of Bakır et al. [19] reported 8.5% pneumococcal carriage rate and 28.8% penicillin resistance in 1,382 healthy children aged between 6 months and 10 years. Uzuner et al. [20] reported those rates as 37.2% carriage rate, 5.4% high-level and 33.9% mid-level penicillin resistance respectively in children aged between 9 days and 67 months. The rate of pneumococcal carriage detected in our study (2.8%) is lower than the results of Uzuner [20], Ciftci [5], Bakır [19] and Gazi [18]. This may be due to the fact that 91.5% of the children included in the present study were vaccinated and no pneumococcal carriage was detected in the vaccinated group. Although the rate of mid-level (33.3%) resistance to penicillin in the isolated strains in our study were similar to previously reported rates of resistance, the rate of high-level resistance to penicillin in our study was apparently higher than the previously reported rates from our country. Low number of isolated strains is a limiting factor in our study, so this point must be taken in to account while evaluating resistance rates.

Nasopharyngeal pneumococcal carriage and the rates of resistance to penicillin show variation according to country or region. While in a study involving the different residential areas of the United States [21] pneumococcal carriage and the rates of penicillin-resistance were 4-9% and 14-24% (high-level) respectively, in a study conducted in Russia [22] 45-66% carriage rate and 7.5% (mid-level) resistance rate was reported. Quite different results were reported about the rates of mid and high level resistance to penicillin; 13.1% mid-level and 1.2% high-level resistance in China and 30% mid-level and 41% high-level resistance in Taiwan [23, 24]. In our study, we found higher high-level and mid-level penicillin resistance rates than those reported rates.

While it was reported that pneumococcus and nasopharyngeal colonization primarily are related to age, other factors are also considered to be effective for colonization [25]. In the studies conducted in Canada and Amsterdam it had been identified that children who attend kindergarten had more infections than children that do not and also nasopharyngeal pneumococcal carriage was more frequent in children who attend kindergarten than children that do not [26, 27]. In a study conducted by Katz et al. [28] in 2003 colonization was detected in 60% of children attending kindergarten in St. Petersburg and it was found

that this rate was highest in children between 3-4 years of age. In the study conducted by Chiou et al. [24] in Taiwan nasopharyngeal swab samples of 2905 children ranged between the age of 2 months to 7 years were taken, *S. pneumoniae* has been isolated in 21% of children and only 29% of these strains were susceptible to penicillin (30% were mid-level resistant and 41% highly resistant). In these studies, the rates of carriage in children have been associated with age, number of siblings, crowded living, socioeconomic status, smoking habits of family members, antibiotic use, and climate, environmental and genetic factors [5, 29-32]. In our study we found a strong relation between pneumococcal carriage and vaccination status. None of the vaccinated children were carriers. The overall rate of pneumococcal carriage rate in our study was lower than most of the previously reported data from both our country and different regions of the world.

Conclusion

This is the first epidemiologic study evaluating the pneumococcal carriage and penicillin resistance after 7-valent pneumococcal conjugate vaccination programme has started in our country. Nasopharyngeal pneumococcal carriage rate in our study was 2.8% in preschool children aged between 4 and 6 years. Carriage rate decreased very seriously from 35% to 2.8%. In our study, among different factors investigated, vaccination status is the only variable associated with nasopharyngeal carriage. This low carriage rate may be the expected result of vaccination nationwide. Despite low pneumococcal colonization rate, we found an increased rate of high-level penicillin resistance in the isolated strains. Since the number of isolated strains is low (n=6) in our study, we think that our findings highlight increased penicillin resistance should be supported by multi-center surveillance studies, so that a national strategy about rising pneumococcal resistance can be established.

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