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The Investigation of Nasal MRSA Carriage and Colonization of Nasopharyngeal Pathogens at a Primary School in Düzce

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Aim: The aim of the study was to investigate nasopharyngeal colonization of respiratory tract pathogens and nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in healthy schoolchildren (6-14 years old).

Materials and Methods: The present study was conducted at Konuralp Primary School by Düzce University and 484 healthy children were included. Isolates obtained from nasal and nasopharyngeal cultures were identified by conventional microbiological procedures and Api 20 strep, NH.

Results: 56.6% of healthy schoolchildren had at least one nasopharyngeal potential bacterial pathogen. Isolated bacteria species were as follows: 6% group A streptococcus (GAS), 5.0% *S. pneumoniae*, 33.1% *M. catarrhalis*, and 34.9% *H. influenzae*. All of the isolated GAS species were susceptible to penicillin. 8.3% of *S. pneumoniae* isolates were intermediately resistant to penicillin. Beta-lactamase test was found positive for *M. catarrhalis* and *H. influenzae* in 90.3 and 8.3%, respectively. There was a significant relationship between nasopharyngeal colonization of *M. catarrhalis* and antibiotic usage in the past six months (P=0.018) and nasopharyngeal colonization of *H. influenzae* and antibiotic usage in the household (P=0.001). The rate of nasal MRSA carriage in healthy children was 5%. This rate was found higher in the 6-10 age group compared to the 11-14 age group (P=0.012).

Conclusions: Our data showed that nasopharyngeal carriage of potential respiratory pathogens in schoolchildren should not be underestimated. More comprehensive surveillance studies should be performed to obtain correct information about the carriage.

Key Words: Nasal MRSA carriage, nasopharyngeal colonization, respiratory tract pathogens, schoolchildren

Düzce'de Bir İlköğretim Okulunda Nasal MRSA Taşıyıcılığı ve Nazofaringeal Patojenlerin Kolonizasyonunun Araştırılması

Amaç: Bu çalışmanın amacı sağlıklı okul çocuklarında (6-14 yaş) solunum yolu patojenlerinin nazofaringeal kolonizasyonu ile nasal methicillin-resistant *Staphylococcus aureus* (MRSA) taşıyıcılığının araştırılmasıdır.

Yöntem ve Gereç: Bu çalışma Düzce Üniversitesi tarafından Konuralp ilköğretim okulunda 484 sağlıklı çocukta yapılmıştır. Kültürlerden elde edilen izolatlar klasik mikrobiyolojik yöntemler ve Api 20 strep, NH ile tiplendirildi.

Bulgular: Sağlıklı okul çocuklarının %56,6'sında en az bir potansiyel nazofaringeal bakteriyel patojen saptandı. İzole edilen bakterilerin %6'sı grup A streptococcus (GAS), %5,0'i *S. pneumoniae*, %33,1'i *M. catarrhalis* ve %34,9'u *H. influenzae* idi. İzole edilen GAS türlerinin tamamı penisiline duyarlıydı. *S. pneumoniae* izolatlarının %8,3'ü penisiline orta düzeyde dirençliydi. Beta-laktamaz testi *M. catarrhalis* ve *H. influenzae* için sırasıyla, %90,3 ve %8,3 pozitif bulundu. *M. catarrhalis*'in nazofaringeal kolonizasyonu ile son 6 ay içinde antibiyotik kullanımı (P=0.018) ve *H. influenzae*'nın nazofaringeal kolonizasyonu ile ev içinde antibiyotik kullanımı (P=0.001) arasında anlamlı bir ilişki saptandı. Nazal MRSA taşıyıcılığı sağlıklı çocuklarda %5 bulundu. Bu oran 6-10 yaş grubunda 11-14 yaş grubuna göre daha yüksekti (P=0.012).

Sonuç: Okul çocuklarında potansiyel solunum yolu patojenlerinin nazofaringeal taşıyıcılığını göz ardı edilmemelidir ve bu konuda daha gerçekçi bilgilerin elde edilebilmesi için daha kapsamlı sürveyans çalışmaları yapılmalıdır.

Anahtar Sözcükler: Nazal MRSA taşıyıcılığı, nazofaringeal kolonizasyon, solunum yolu patojenleri, okul çocukları

Introduction

The normal microbial flora, although important for the maintenance of human health, can play a critically important role in infectious diseases. It is reported that physicians see more patients with infectious diseases acquired from endogenous microbial flora than from exogenous sources (1).

Microbial colonization of the nasopharynx occurs soon after birth, following aerosol exposure or spread of respiratory tract microorganisms from those individuals in close contact with the infant. The normal microbial flora of the infant establishes itself within several months and generally remains unchanged throughout life (2). The nasopharynx has a flora similar to that of the mouth and is the site of carriage of potentially pathogenic bacteria such as *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* (2).

The external 1 cm of the external nares is lined with squamous epithelium and has a flora similar to that found on the skin, except that *Staphylococcus aureus* is commonly carried as the principal part of the normal flora in some individuals. Approximately 25 to 30% of healthy people in the community harbor this organism in their anterior nares at any given time, 15% permanently and 15% transiently (2).

The aim of the study was to investigate the nasal carriage of *S. aureus* and to determine the nasopharyngeal carriage of *S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis,* and group A beta hemolytic streptococcus (GAS) in healthy schoolchildren (6-14 years old).

Materials and Methods

Setting and Study Population

Düzce is a city famous for its wood industry and agriculture. It has a 20 kilometer square land area and 328,000 population overall. There are also medium level industrial institutions. It is 30 km inland from the western Black Sea coast. The primary school in which our study was conducted is located in the territory which was formerly a Roman Empire settlement.

The present study was conducted at Konuralp Primary School in Düzce in autumn by Düzce University and included 484 healthy children (257 female, 227 male, age range: 6 to 14 years, mean age: 10.1±2.21). All participants were examined by an infectious disease specialist. Children with any infection and using antibiotics during the last two weeks were excluded from the study. Antibiotic usage in the last six months and history of hospitalization were recorded. A short questionnaire regarding the demographic characteristics of the study group such as age, gender, antibiotic usage in the past six months, history of hospitalization, and antibiotic usage in the household was administered to all participants.

Specimens and Procedures

All children underwent nasopharyngeal and nasal cultures. For the investigation of nasal *S. aureus* carriage, a specimen was obtained from the anterior nares of the nose. Mouthwashing technique was used for taking nasopharyngeal samples (3). The samples were obtained from the participants and processed within 2-3 h of collection. Colonization of GAS, *S. pneumoniae, H. influenzae, M. catarrhalis* and *S. aureus* in the upper respiratory tract was investigated. Each swab was inoculated on to a variety of aerobic bacteriological culture media, which were then incubated in an appropriate atmosphere. The specimens were inoculated for *M. catarrhalis* isolation to selective media as recommended by Vaneechoutte et al. (4). The medium was prepared by adding 10 mg/L vancomycin, 5 mg/L of trimethoprim, 2 mg/L amphotericin B and 10 mg/L of acetazolamide to trypticase soy agar and supplemented with 5-7% sheep blood. In addition, specimens were inoculated on blood and chocolate agar for other microorganisms. *M. catarrhalis* selective medium and blood agar were incubated aerobically, whereas chocolate agar was incubated in a candle jar containing 5-10% CO2. Plates were examined after 24 and 48 h of incubation aerobically at 37°C. A semiquantitative measure of growth was made for all colony types. Each colony type was recorded and subcultured. Isolates obtained on subculture plates were identified by conventional microbiological procedures and Api 20 strep, NH (bioMerieux, Marcy-l'Etoile, France). Antimicrobial susceptibility testing of all isolates against oxacillin with agar screening method and other antibiotics with disc diffusion method with Mueller-Hinton agar (bioMerieux, Marcy-l'Etoile, France) was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (5). Nitrocefin test (Becton Dickinson Diagnostic Instruments, USA) was employed for detection of beta-lactamase activity in *H. influenzae* and *M. catarrhalis* isolates. The capsular antigenic typing of *H. influenzae* was done by slide coagglutination (Phadebact; Polygon Diagnostics, Switzerland).

Statistical Analysis

SPSS 11.0 for Windows program was used for statistical analysis. Relationships between antimicrobial

resistance/colonization rates of respiratory pathogens and risk factors such as age, gender, antibiotic usage in the past six months, history of hospitalization, and antibiotic usage in the household were evaluated by a logistic model formed from a multi-nominal logistic regression analysis. Pearson chi-square test was used to compare oxacillin resistance rates of *S. aureus* isolates and nasal methicillin-resistant *S. aureus* (MRSA) carriage rates according to age groups (6-10 and 11-14 age groups). Mupirocin resistance rates in MRSA and methicillin-sensitive *S. aureus* (MSSA) isolates were also evaluated by Pearson chi-square test. The level of significance for statistical tests was $P<0.05$.

Results

Nasopharyngeal Results

Nasopharyngeal cultures were taken from 484 children to determine the carriage of *S. pneumoniae, H. influenzae, M. catarrhalis,* GAS, and *S. aureus*. Two hundred and seventy four (56.6%) healthy schoolchildren had at least one potential bacterial pathogen, 189 (39%) had only one bacterium, 64 (13.2%) had two bacteria and 21 (41%) had more than two bacteria in nasopharyngeal flora. Isolated bacteria species were as follows: 29 (6%) GAS, 24 (5.0%) *S. pneumoniae*, 160 (33.1%) *M. catarrhalis*, and 169 (34.9%) *H. influenzae*. Of 169 isolates of *H. influenzae*, 13 (8.0%) were serotype b and 156 (92%) non-type b.

Distribution of the isolates according to age groups is shown in Table 1. All of the isolated GAS species were susceptible to penicillin and only one (3.4%) was resistant to erythromycin. Two (8.3%) isolates of *S. pneumoniae* were intermediately resistant to penicillin. Beta-lactamase test was found positive for *M. catarrhalis* and *H. influenzae* in 90.3 and 8.3%, respectively.

There was a significant relationship between nasopharyngeal colonization of *M. catarrhalis* and antibiotic usage in the past six months (P=0.018, OR: 1.873, CI95%: 1.113-3.154). There was a significant relationship between nasopharyngeal colonization of *H. influenzae* and antibiotic usage in the household (P=0.001, OR: 1.953, CI95%: 1.307-2.917). There was no relationship between nasopharyngeal colonization of *M. catarrhalis* and *H. influenzae* and age, gender or history of hospitalization (P>0.05). No relationship was found between nasopharyngeal colonization of GAS and *S. pneumoniae* and the risk factors (P>0.05).

Nasal Results

S. aureus was isolated from 179 (37.0%) of 484 nasal cultures in healthy children; 5% of the children (24/484) had MRSA. Distribution of *S. aureus* isolates according to age groups is shown in Table 2. The penicillin resistance of all *S. aureus* isolates was 95.0% (170/179), oxacillin resistance was 13.4% (24/179) and mupirocin resistance was 36.9% (66/179). Nasal MRSA carriage was found in 7.3% (19/262) in the 6-10 age

Table 1. Distribution of nasopharyngeal isolates according to age groups.

Age (n =)	GAS		*S. pneumoniae*		*M. catarrhalis*		*H. influenzae*	
	n	%	n	%	n	%	n	%
6 (27)	2	7.4	2	7.4	8	29.6	13	48.1
7 (52)	4	7.7	2	3.8	17	32.7	19	36.6
8 (39)	3	7.7	4	10.3	16	41.0	18	46.2
9 (72)	4	5.6	2	2.8	27	37.5	24	33.3
10 (72)	3	4.2	5	6.9	26	36.1	23	31.9
11 (65)	3	4.6	4	6.2	22	33.8	19	29.2
12 (86)	4	4.7	3	3.5	18	20.9	22	25.6
13 (56)	3	5.4	2	3.6	15	26.8	20	35.7
14 (15)	3	20.0	0	0.0	11	73.3	11	73.3
Total (484)	29	6.0	24	5.0	160	33.1	169	34.9

Table 2. Nasal carriage of *S. aureus* according to age groups.

Age Groups	MSSA		MRSA		Total	
	n	%	n	%	n	%
6 (27)	9	33.3	2	7.4	11	40.7
7 (52)	14	26.9	3	5.8	17	32.7
8 (39)	11	28.2	3	7.7	14	35.9
9 (72)	18	25.0	5	6.9	23	31.9
10 (72)	21	29.2	6	8.3	27	37.5
11 (65)	27	41.6	2	3.1	29	44.6
12 (86)	31	36.0	2	2.3	33	38.4
13 (56)	16	28.6	1	1.8	17	30.4
14 (15)	6	40.0	0	0.0	6	40.0
Total (n=484)	155	32.0	24	5.0	179	37.0

group and in 2.3% (5/222) in the 11-14 age group, and the difference between the two groups was significant (P=0.012).

There was no relationship between *S. aureus* nasal carriage and demographic characteristics such as age, gender, antibiotic usage in the past six months, history of hospitalization and antibiotic usage in the household (P>0.05).

Oxacillin resistance of *S. aureus* isolates in the 6-10 age group was found higher compared to the 11-14 age group (P=0.003). There was no relationship between oxacillin resistance rates and risk factors such as gender, antibiotic usage in the past six months, history of hospitalization and antibiotic usage in the household (P>0.05). There was also no relationship between mupirocin resistance and risk factors (P>0.05). The relation between oxacillin and mupirocin resistance and risk factors is shown in Table 3.

Mupirocin resistance was found higher in MRSA isolates (83.3%) than MSSA isolates (29.7%) (P<0.001). Distribution of *S. aureus* isolates according to mupirocin resistance is shown in Table 4.

Table 3. Relationships between oxacillin and mupirocin resistance of *S. aureus* and risk factors.

Risk factors			Oxacillin Res			Mupirocin Res		
			n	%	P	n	%	P
Age	6-10	(92)	19	20.7	0.003*	36	39.1	0.413
	11-14	(85)	5	5.9		29	34.1	
Gender	Male	(93)	10	10.8	0.129	38	40.9	0.302
	Female	(86)	14	16.3		28	32.6	
Antibiotic usage in the past 6 months	Yes	(33)	6	18.2	0.490	16	48.5	0.094
	No	(105)	13	12.4		35	33.3	
History of hospitalization	Yes	(43)	7	16.3	0.483	16	37.2	0.710
	No	(96)	12	12.5		34	35.4	
Antibiotic usage in the household	Yes	(62)	9	14.5	0.681	17	27.4	0.121
	No	(90)	12	13.3		36	40.0	

* Relationship between oxacillin resistance and age: OR: 0.182, 95%CI: 0.058-0.565.

Table 4. Mupirocin resistance in *S. aureus* isolates.

Microorganism	Mupirocin sensitive		Mupirocin resistant		
	n	%	n	%	Total
MSSA	109	70.3	46	29.7	155
MRSA	4	16.7	20	83.3	24
Total	113	63.1	66	36.9	179

X^2: 25.7, P<0.001

Discussion

The normal microbial flora of the infant establishes itself within several months and generally remains unchanged throughout life (2). The nasopharynx has a flora similar to that of the mouth and is the site of carriage of potentially pathogenic bacteria such as *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* (2). They are the most important bacterial pathogens causing mucosal infections such as otitis media, sinusitis, bronchitis and meningitis (6). In the present study, 274 (56.6%) healthy schoolchildren had at least one potential bacterial pathogen, 189 (39%) only one bacterium, 64 (13.2%) two bacteria, and 21 (41%) more than two bacteria in nasopharyngeal flora.

S. pneumoniae is an important agent of otitis media, sinusitis, meningitis, and community-acquired pneumonia that may be accompanied by bacteremia. Oropharyngeal carriage of pneumococci is common and contributes to the difficulty of interpreting the significance of pneumococci in cultures of expectorated sputum (7). In the present study, *S. pneumoniae* carriage rate was 5.0% in healthy children. The rates of nasopharyngeal carriage of *S. pneumoniae* have been reported to vary from 2.9 to 99% (6,8-10).

H. influenzae colonizes the upper respiratory tract of humans. In addition to asymptomatic colonization, *H. influenzae* also causes significant infection (11). In the present study, *H. influenzae* carriage rate was 34.9% in healthy children. The rates of nasopharyngeal carriage of *H. influenzae* have been reported to vary from 5 to 87% (2,8-10,12).

M. catarrhalis causes mucosal infections in children and adults. The pathogenesis of infection appears to involve contiguous spread of the bacterium from its colonizing position in the respiratory tract to cause clinical signs of infection (13). The nasopharyngeal colonization rates of *M. catarrhalis* were reported as 1 to 74% in the literature (8-10,14). Substantial regional differences in colonization rates are observed. For example, 66% of infants in a study in Buffalo, New York were colonized during the first year of life (15), whereas a similar study in Goteberg, Sweden showed a colonization rate of approximately half that level (16). In our study, we found nasopharyngeal colonization rate of *M. catarrhalis* as 33.1% in healthy children.

Streptococcal sore throat is among the most common bacterial infections of childhood. GAS is responsible for the great majority of such infections. The disease occurs primarily among children 5 to 15 years of age, with the peak incidence occurring during the first few years of school. GAS frequently colonizes the throats of asymptomatic persons. Pharyngeal carriage rates among normal schoolchildren vary with the geographic location and season of the year (especially in winter) (17). Limura et al. (18) found GAS colonization rate as 15.8% among 20118 healthy children over the last 20 years. Millar et al. (12) reported the incidence of GAS carriage as 3.3%. In the study of Begovac et al. (19), the incidence of GAS carriers was found as 6% among healthy children. In our study, we found the rate of nasopharyngeal colonization of GAS as 6% in healthy children, which was similar to the rates in other studies.

Respiratory tract infections among children are a common reason for health care provider visits and the primary reason for antimicrobial prescriptions in this population. The increased prevalence of resistance among *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* pathogens poses a serious challenge in the successful treatment of respiratory tract infections caused by these

pathogens (20). Inoue et al. (21) reported that penicillin resistance was high among isolates of *S. pneumoniae*, averaging 30.9-44.5% across all centers; beta-lactamase production among *H. influenzae* isolates ranged between 8.5%-9.7% per annum. Almost all (>95%) *M. catarrhalis* isolates were beta-lactamase positive each year. In the present study, two (8.3%) isolates of *S. pneumoniae* were intermediately resistant to penicillin. All of the isolated GAS species were susceptible to penicillin and only one (3.4%) was resistant to erythromycin. Beta-lactamase test was found positive for *M. catarrhalis* and *H. influenzae* in 90.3% and 8.3%, respectively. These results suggest that pediatric bacterial isolates are often resistant to various antimicrobial agents, and this higher resistance rate may be due to frequent use of antimicrobial treatments in children and extensive child to child transmission.

S. aureus is a frequent cause of infections in both the community and hospital. Worldwide, the increasing resistance of this pathogen to various antibiotics complicates treatment of *S. aureus* infections. Effective measures to prevent *S. aureus* infections are therefore urgently needed. It has been shown that nasal carriers of *S. aureus* have an increased risk of acquiring an infection with this pathogen (22). The prevalence of nasal *S. aureus* carriage has been reported to vary from 10% to 36.4% (23-26); nasal carriage rates for MRSA were found as 1.9-15% in healthy children (24,26,27). In our study, nasal *S. aureus* and MRSA carriage was 37% and 5% (7.3% in 6-10 age group and 2.3% in 11-14 age group, P=0.012) in healthy children, respectively. Our results were compatible with other studies. The fact that nasal MRSA carriage was found higher in the 6-10 age group may be related to the frequent antibiotic usage in this age group in Turkey.

Mupirocin is used primarily in skin infections, which are usually caused by *S. aureus* and *Streptococcus pyogenes*. Mupirocin can also eliminate nasal carriage of *S. aureus.* The development of mupirocin resistance in MRSA has emerged as a problem after widespread use of nasal mupirocin (28). In one study, mupirocin resistance among MRSA after the use of nasal mupirocin increased from 2.7 to 65% from 1990 to 1993 (29). The mupirocin resistance rate of MRSA strains was observed as 63% in another study (30). In the present study, the mupirocin resistance of all *S. aureus* isolates and of MRSA isolates in healthy children was 36.9% and 83.3%, respectively. The fact that mupirocin resistance was found high (P<0.001) especially in MRSA isolates suggests that eradication of nasal carriage would be difficult in children with mupirocin usage. Hence, conventional infection control procedures such as handwashing should be used for preventing nasal carriage of *S. aureus*.

In conclusion, our data showed that nasopharyngeal carriage of potential respiratory pathogens in schoolchildren should not be underestimated. There were significant relationships between nasopharyngeal colonization of *M. catarrhalis* and antibiotic usage in the past six months, and between nasopharyngeal colonization of *H. influenzae* and antibiotic usage in the household. No relationship was determined between nasopharyngeal colonization and age, gender and history of hospitalization. *S. aureus* was isolated from 37% of nasal cultures in healthy children; 5% of all the children had MRSA. The high prevalence of *S. aureus* and MRSA nasal colonization in healthy children suggests the need for appropriate antimicrobial use to halt or at least limit the spread of resistance. More comprehensive surveillance studies should be performed to obtain correct information about the carriage rate and antimicrobial resistance of these pathogens in schoolchildren.

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