

THE EFFECT OF DAY CARE CENTRES TO NASOPHARYNGEAL STREPTOCOCCUS PNEUMONIAE COLONIZATION AND PENICILLIN RESISTANCY

GÜNDÜZ BAKIM EVLERİNİN NAZOFARINGEAL STREPTOCOCCUS PNEUMONIAE KOLONİZASYONU VE PENİSİLİN DİRENCİNE ETKİSİ

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Özet

The aims of the present study were to investigate the prevalence of nasopharyngeal carriage of Streptococcus pneumoniae and to determine the antibiotic resistance patterns and serotypes in healthy children attending day care centre at 0, 3 and 6th months. A total of 295 healthy children (age ranged: 4-7) attending the five day care centres were included to the study. The isolation and identification of S. pneumoniae strains from nasopharyngeal samples were performed by conventional culture methods. Antibiotic sensitivity tests were done according to the Clinical Laboratory Standards Institute directions by disk diffusion method, and penicillin MIC values were detected by microdilution method. S. pneumoniae were isolated 18.6% (55) children at the first day attended the day care but nasopharyngeal carriage were reached 34.6% at the third month, 47.1% at the sixth month. The susceptibility rate of the isolates to penicillin was found as 68.7%, while 10.2% of the strains yielded high resistance against penicillin. Overall percentages of resistance to rifampicin, erythromycin and clindamycin were 16.3%, 23.5% and 9.6%, respectively. Multidrug resistant S. pneumoniae was detected in 8 (4.8%) of strains. The most frequent serotypes associated with penicillin resistance were serotypes 6, 8, 9, 15, 19, 20 and 23. Finally, this study shows that staying in day-care centres is very important risk factors for S. pneumoniae carrier state. Due to most isolated and penicillin resistant strains covered by 7-valent vaccine, suggesting that its use could reduce the incidence of pneumococcal disease. (Anatol J Clin Investig 2010;4(1):11-14).

Abstract

Bu çalışmanın amacı, çocuk yuvasında sağlıklı çocukların yuvaya kabul edildiği ve sonrasındaki üçüncü ve altıncı aylardaki nazofaringeal Streptococcus pneumoniae taşıyıcılık oranlarının belirlenmesi ve bunun yanında izole edilen suşların antibiyotik duyarlılığı ve serotiplerinin ortaya konmasıdır. Çalışmaya beş çocuk yuvasında 295 sağlıklı çocuk dahil edildi. Nazofaringeal örneklerden S. pneumoniae izolasyon ve identifikasyonu konvansiyonel kültür yöntemleri ile yapıldı. Antibiyotik duyarlılık testleri Klinik ve Laboratuvar Standartları Enstitüsü standartlarına göre yapılmıştır. S. pneumoniae yuvaya katılışın yapıldığı günde olguların %18,6'ında izole edilirken üçüncü ayda bu oran %34,6'ya altıncı ayda ise %47,1 oranına ulaşmıştır. İzolatların %68,7'si penisiline duyarlı iken %10,2'sinde yüksek düzeyde penisilin direnci saptanmıştır. Rifampisin, eritromisin ve klindamisin direnci ise sırasıyla %16,3, %23,5 ve %9,6 oranında saptanmış olup izolatların %4,8'inde çoklu ilaca direnç saptanmıştır. Penisilin direnci en sık serotip 6, 8, 9, 15, 19, 20 ve 23'te saptanmıştır. Sonuç olarak çocuk yuvaları S. pneumoniae taşıyıcılığına etki eden önemli bir risk faktörüdür. İzole edilen ve penisiline dirençli serotiplerin çoğunluğu 7 valanlı konjüge aşı içeriğinde bulunduğundan, bu aşının önerilmesi pnömokokal hastalık insidansında azalmayı sağlayacaktır. (Anatol J Clin Investig 2010;4(1):11-14).

Introduction

Streptococcus pneumoniae is one of the most important agents of serious infections observed in children. In recent years, emergence of penicillin-resistant *S. pneumoniae* strains has made it difficult to treat the infections caused by these organisms. Although nasopharyngeal colonization plays an important role for infections of *S. pneumoniae*, the factors affecting the nasopharyngeal colonization with or without penicillin-resistant *S. pneumoniae* have not been understood.

The potential risk factors for *S. pneumoniae* carriage are well studied and include having day

care centre and family contacts, young age, previous antibiotic consumption, underlying disease, living with smokers and recent respiratory tract infection [1]. Although a previous study revealed that seasonal variation did not have an important effect on nasopharyngeal carriage of *S. pneumoniae*. The frequency of carriers was significantly increased in colder months in a hot tropical country (India) [2].

There is a continuous increase in antibiotic resistance among *S. pneumoniae* isolates over the world, reflecting circulation of resistant stains in the community. Emergence of penicillin-and

multidrug resistant strains even in healthy carriers may complicate empirical treatment of infections due to such strains. *S. pneumoniae* strains are divided into more than 90 serotypes. Serotyping and drug susceptibility tests should be performed periodically to detect resistance and serotype distribution in a population, which might be helpful for appropriate empirical antibiotic therapy and design of new strategies in vaccine preparation [3].

Data regarding the frequency of carriers, antimicrobial resistance, serotypes and clonally relatedness of *S. pneumoniae* strains isolated from children in Turkey are lacking. The aims of the present study were to investigate the rate of nasopharyngeal colonisation, antimicrobial susceptibility, serotypes and clonal relatedness of *S. pneumoniae* strains isolated from healthy children in day care centres at 0, 3 and 6th months.

Material and methods

This study was carried out on 295 children from five different day care centres in Istanbul. The age of children attending day care centres generally ranges from 4-7 years old. These day care centres were selected according to their socio-economic status, which could represent the general characteristics of the school children in the city. Children who fulfilled the following criteria were included in the study (1) no signs of respiratory infection and no antibiotic exposure within the last 2 weeks; (2) they had obtained permission from their parents; and (3) they were in the first to sixth month of these day care centres. Permission was obtained from the local Ethical Committee of the Gulhane Military Medical Academy.

A single swab was obtained over the posterior nasopharyngeal walls of the 295 apparently healthy children. Bacterial identification was performed by classic biochemical method. The disk diffusion method was performed on Mueller-Hinton agar (Oxoid Ltd, Basingstoke, UK) with 5% sheep's blood in accordance with the guidelines of the Clinical Laboratory Standards Institute. Penicillin resistance was screened with 1 microgram oxacillin disk (Oxoid Ltd, Basingstoke, UK). Isolates showing inhibition zones ≤ 19 mm were confirmed by the microdilution method. Breakpoints used for interpretation of minimum inhibitory concentrations (MICs) were ≤ 0.06 mg/L (susceptible), 0.1-1.0 mg/L (intermediate) and ≥ 2.0 mg/L (resistant). Antibacterial susceptibility was also detected for levofloxacin, rifampicin, erythromycin, clindamycin and telithromycine.

The serotype of the isolated *S. pneumoniae* strains were detected by Quellung reaction using 12 pooled Pneumotest antisera covering vaccine serotypes (Staten Serum Institute, Copenhagen, Denmark). For better visualisation, 1% methylene blue was used.

Results

Totally 295 children with appropriate criteria of 410 children were included in this study. In the first period carrier state of *S. pneumoniae* was identified in 55 children (18.6%), in 102 (34.6%) children in the second period and 139 (47.1%) children in the third period ($p=0.001$ vs. $p=0.001$). Comparison of the colonization rate among the day care centres are presented in table. There was statistically significant increase in the colonization rate in Selimiye Day Care Centre ($n=84$) ($p=0.001$), in Selim III. Day Care Centre ($n=64$) ($p=0.004$), and Esentepe Day Care Centre ($n=81$) ($p=0.004$), compared with first and second period. The increasing trend was found in Sarıköşk Day Care Centre ($n=43$) ($p=0.001$), Selim III. Day Care Centre ($p=0.0001$), and Esentepe Day Care Centre ($p=0.001$) compared with the first and third period. On the other hand, the increasing trend was found in Sarıköşk Day Care Centre ($p=0.001$), Selimiye Day Care Centre ($p=0.012$), and Esentepe Day Care Centre ($p=0.004$) compared with the second and third period. The increase in the Gamze Day Care Centre in third period was not statistically significant ($p>0.01$).

We found that 31.3 % (52) of the *S. pneumoniae* isolates defined as penicillin resistant by oxacillin disk and 10.2% (17) of those defined as high level penicillin resistant strain by microdilution method. Antibacterial resistance was also detected in 27 (16.3%) strains for rifampicin, in 39 (23.5%) strains for erythromycin, and in 16 (9.6%) strains for clindamycin. No strains were found as resistant to fluoroquinolones and telithromycine. Multidrug -resistant *S. pneumoniae* was detected in 8 (4.8%) of strains. The most frequent serotypes associated with penicillin resistance were serotypes 6, 8, 9, 15, 19, 20 and 23, which covered by 23-valent and 7-valent conjugated pneumococcal vaccine.

Discussion

Living in day care centres is the one of the most important risk factor for nasopharyngeal carriage of *S. pneumoniae*. Higher rates (more than 60%) were also found in day care centres in which many children stay together for a long period [4]. This study is the first report reflecting the carriage status of *S. pneumoniae* among the children increases during the living period. We also found that the overall rate of

nasopharyngeal *S. pneumoniae* carriers varies among different day care centres in Istanbul. The colonisation rates were found 38.3-62.8%. Difference might be related to social and living condition. Day care centres which found high colonisation rates in localised poor socioeconomic area. Generally, families in slum areas have many children living together in small houses, therefore they are at risk of becoming colonised and this overcrowding may be a risk factor for high carriage.

In this study, we found that 31.3% of the *S. pneumoniae* isolates defined as penicillin resistant by oxacillin disk and 10.2% of those defined as high level resistant to penicillin by microdilution method. All strains with an oxacillin inhibition zone diameter of ≤ 14 mm were found to be resistant to penicillin. Because the oxacillin screening method can not distinguish intermediate and high resistant strains, as indicated previously, microdilution method was the most accurate, cheap and effective method for determination of penicillin resistance in *S. pneumoniae* isolates.

Multidrug resistant *S. pneumoniae* is still a big problem throughout the world [5]. Recent studies have indicated that this problem was increasing especially in children population [5,6]. We observed that 4.8% of the children have multidrug resistant. Close contact among children and frequent antibiotic treatment favour the selection and spread of resistant pneumococci. In the present study, high level penicillin resistant strains were increasing in the day care population. For this reason,

telithromycine may be alternative choice of treatment instead of penicillin in day care children.

Previous studies indicated that certain serotypes are more resistant or prevalent than others. Many investigators showed that serotypes 6, 9, 14, 19 and 23 were the most frequent ones [7,8]. In agreement with these data, serotypes 6, 8, 9, 15, 19, 20 and 23 were found predominate serotypes among the high level penicillin resistant *S. pneumoniae* isolates in our area, and serotypes 9 and 23 were most common isolates both among penicillin susceptible and non-susceptible isolates.

Serotyping is of paramount importance in planning new strategies for vaccine development, which can decrease and prevent the spread of pneumococcal infections and reduce mortality. Serotypes and resistance patterns found in the current study may help to prepare an appropriate pneumococcal vaccine in our location. For this reason, there will be a need for surveillance of resistance and our serotype distribution, which covered by 23 valent and 7-valent conjugated pneumococcal vaccines, in our geographical area from time to time.

Conclusion

This study shows that staying in day-care centres is very important risk factors for *S. pneumoniae* carrier state. Most isolated strains are covered by 7-valent conjugate vaccine, suggesting that its use could reduce the incidence of pneumococcal disease.

Table 1. Comparison of the colonization rate among the day care centres

Day care Centre (n)	I. Period		II. Period		III. Period		Comparison of periods (p)		
	Strains (n)	%	Strains (n)	%	Strains (n)	%	I-II	II-III	I-III
Sarıköşk (43)	16	37.2	16	37.2	27	62.8	1	0.001	0.001
Gamze (23)	8	34.7	9	39.1	12	52.8	1	0.25	0.12
Selimiye (84)	11	13.1	33	39.2	42	50.0	0.001	0.012	0.0001
III. Selim (64)	11	17.2	23	35.4	28	43.7	0.004	0.12	0.0001
Esentepe (81)	9	11.1	21	25.9	30	38.3	0.004	0.004	0.001
Total (295)	55	18.6	102	34.6	139	47.1	0.001	0.001	0.0001

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