

Prevalence of Group A Streptococcal Carriers in Asymptomatic Children and Clonal Relatedness among Isolates in Malatya, Turkey

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In our study, the prevalence of nasopharyngeal *Streptococcus pyogenes* was 130 (14.3%) of 909 healthy children. Isolates were found to be susceptible to all antibiotics tested. Pulsed-field gel electrophoresis and arbitrarily primed PCR revealed that 34 (32.4%) of the 105 isolates and 41 (40.6%) of the 101 isolates typed, respectively, were clonally indistinguishable.

Streptococcus pyogenes group A streptococcus (GAS) strains colonized in the upper respiratory tracts of children play an important role in the spread of this bacterial infection, especially among children at school, day-care centers, orphanages, and home. Study of the prevalence of healthy *S. pyogenes* carriers and the molecular epidemiology of the isolates may provide useful information about the origin and spread of this infectious agent, allowing for more effective control measures. Pulsed-field gel electrophoresis (PFGE) (3, 4, 14) has been used as a standard technique for surveying epidemiology of *S. pyogenes* infections. Although arbitrarily primed PCR (AP-PCR)-based fingerprinting performed with the M13 primer has been widely used for molecular epidemiology of gram-negative (1, 2, 10) and gram-positive bacteria (11), there had been no study about its efficiency in typing *S. pyogenes* strains.

The aims of the present study were to investigate the rate of pharyngeal colonization, drug susceptibility, and the molecular epidemiology of GAS isolated from healthy children and to compare PCR-based fingerprinting with PFGE in the investigation of clonal relatedness among the GAS isolates.

Study groups. The study groups included 800 primary schoolchildren and 109 children living in an orphanage in Malatya, Turkey. An otorhinology specialist rubbed sterile swabs over the posterior nasopharyngeal walls of the 909 children, who had no symptoms or signs of pharyngitis. The samples were inoculated on sheep's blood agar plates. After incubation overnight at 37°C, beta-hemolytic streptococci were identified with a bacitracin disk (0.04 U) and a latex test for the identification of streptococcal groups A, B, C, D, F, and G (streptococcal grouping kit and diagnostic reagent; Oxoid Limited, Basingstoke, England).

Susceptibility testing. The antimicrobial susceptibilities of the GAS isolates were investigated by the disk diffusion method according to the criteria of the National Committee for Clinical Laboratory Standards (13). The antibiotic disks

(Oxoid) used were penicillin (10 U), erythromycin (15 µg), vancomycin (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), cefepime (30 µg), ceftriaxone (30 µg), ofloxacin (5 µg), and levofloxacin (5 µg).

Molecular typing of the strains. Both AP-PCR and PFGE typing was performed on 101 strains which had available stocks. PFGE was also carried out on five additional strains. For AP-PCR typing, isolation of DNA by using lysozyme and proteinase K and extraction were performed by following the protocol of Welsh and McClelland (19). Then AP-PCR, which had previously been optimized (1, 2), was performed with the M13 primer (10). For PFGE, isolation and deproteinization of the genomic DNA were done by following the protocol of Elliott et al. (8) but with lysostaphin (5 U/ml) used instead of mutanolysin. The genomic DNA in the plugs was incubated with 24 U of *Sma*I (Promega Corporation, Madison, Wis.) for 24 h at 25°C in a water bath. DNA fragments were separated on 1% agarose gels run in 0.5× Tris-borate-EDTA buffer by using a CHEF-DR II system (Bio-Rad Laboratories, Nazareth, Belgium). The conditions for electrophoresis were 14°C at 6 V/cm for 24 h. The initial and final switch times were 5 and 40 s, respectively. The band patterns of the strains obtained with both typing procedures were analyzed with GelCompar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium). According to the interpretative criteria of Tenover et al. (17), the isolates were classified as indistinguishable (cluster), closely related, possibly related, or different.

The means and standard deviations (SD) were calculated and data were analyzed using the statistical suite SPSS (version 10). The chi-square test was used to determine the significance of difference of the parameters tested.

Analysis of the results. The mean age of the 909 children was 8.17 years (SD, 3.89; range, 4 to 13 years). Of the study group, 491 (54%) were boys and 418 (46%) were girls. The global prevalence of healthy *S. pyogenes* carriers was 14.3% (130 carriers), which was considerably higher than for previous findings (2.5 to 6.8%) (9, 12, 15). Although age, gender, hospitalization during the last 3 months, antibiotic use, sanitary conditions, and being with family members and/or school

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TABLE 1. Frequency of *S. pyogenes* carriers based on study groups

Group	Total no. of children	No. of children who were:		Frequency (%) of carriers in indicated age group			
		Tested (sampling rate %)	Carriers (%)	4-6	7-8	9-10	>10 years
Schoolchildren	1284	800 (62.3)	101 (12.6)	12.1	11.7	13.0	
Orphanage children	135	109 (80.7)	29 (26.6)	50.0	24.0	17.1	15.4
Total	1419	909 (64.1)	130 (14.3)	21.0	13.5	13.3	15.4

workers are risk factors for *S. pyogenes* colonization (16), we found that the rates of carriers for boys and girls were similar (13.6 and 15.1%, respectively; $P > 0.05$) and that there was no significant difference between the mean age of the carriers (7.74 years; SD, 1.91) and that of the noncarriers (8.23 years; SD, 4.13). The frequency was similar in all age groups of schoolchildren ($P > 0.05$), but it was significantly higher in children aged 4 to 6 years living in the orphanage ($P < 0.05$) (Table 1). The prevalence of carriers was significantly higher in the orphanage ($P < 0.001$), suggesting that group living for a long period has an important effect on the spread of GAS.

Similar to the results of previous studies (5, 7, 12), all GAS tested in the present study were susceptible to first-line drugs such as penicillin and erythromycin; moreover, we did not find any strains resistant to other antibiotics tested. These results show that even upper respiratory tract cultures yield *S. pyogenes*. Susceptibility testing is not necessary.

Although many investigators use the AP-PCR method in combination with PFGE for investigation of molecular epidemiology of GAS in a community (4, 5, 6, 18), to date there have been no published data on the clonal diversity of *S. pyogenes* isolates obtained from asymptomatic carriers or symptomatic patients in Turkey. In our study, PFGE yielded 85 different patterns among 105 *S. pyogenes* isolates. Of the isolates, 34 (32.4%) were clustered in 14 groups, 4 (3.8%) were possibly related, and 67 (63.8%) were epidemiologically unrelated. Seventy-seven AP-PCR banding patterns were obtained among 101 isolates. The PCR-based typing procedure revealed that 41 (40.6%) of the isolates were indistinguishable (clustered in 17 groups), 13 (12.9%) were closely related, 12 (11.9%) were possibly related, and 35 (34.6%) were different. The mean numbers of detectable bands obtained by PFGE and AP-PCR were 11.6 (range, 5 to 22) and 10.6 (range, 5 to 21), respectively. Figure 1 provides examples of the band profiles obtained by AP-PCR and PFGE.

A total of 38 (36.1%) and 66 (65.3%) of the isolates typed by PFGE and AP-PCR, respectively, were clonally related. The clonal relationships determined by PFGE (81.6%) and AP-PCR (75.8%) typing confirm conventional epidemiological data that indicate being in the same school and classroom or living in an orphanage increases the prevalence of carriers. When we considered only clustering isolates, concordance between conventional and molecular epidemiology increased to 85.3% for both typing procedures.

Comparison of the typing procedures showed that 76.5% of the strains defined as indistinguishable by PFGE had identical AP-PCR profiles. Thirty-five (92.1%) of the 38 strains defined as clonally related by PFGE also showed clonal relationships by AP-PCR typing. On the other hand, 91.4% of the strains which yielded distinct profiles by AP-PCR were also different by PFGE. Our results revealed that the clustering results of

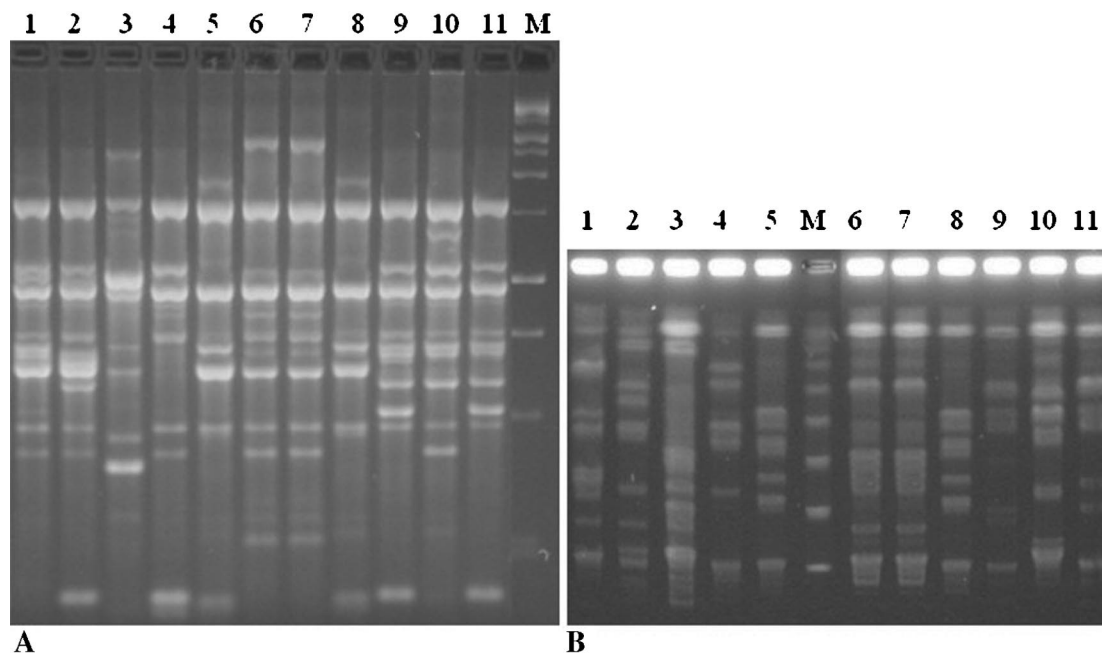


FIG. 1. Representative band patterns of 11 isolates obtained by both typing methods. (A) AP-PCR profiles. Lane M is a molecular weight marker (QX174 DNA/*Hae*III); lanes 6 and 7 are isolates with identical DNA profiles. (B) PFGE profiles. Lane M contains a concatemer of lambda DNA (catalog no. D2416; Sigma); lanes 6 and 7 are isolates with identical profiles.

AP-PCR typing need confirmation; however, strains found distinct by this method most probably do not need retesting.

Molecular typing results showed that there was no association between clustering rate and age and gender of the study populations; however, similar to results for colonization, the isolates of the children in the orphanage had significantly higher clustering rates. As was expected, these data reveal that an increase in time spent with other children leads to an increase in the risk of transmission.

In conclusion, high rates of colonization and clustering obtained by both typing procedures show that the streptococcal populations circulating in children of school age tend to be highly related. The AP-PCR method used in this study is useful in eliminating epidemiologically unrelated isolates.

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