Nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* in healthy children

Arzu Uzuner[1], Arzu İlki[2], Mehmet Akman[1], Ercan Gündoğdu[3], Rıza Erbölükbaş[3]
Ömer Kokaçya[3], Türkan Mengüç[3], Sibel Kalaça[4], Güner Söyletir[2]

Departments of [1]Family Medicine, [2]Microbiology, and [4]Public Health, Marmara University [3]Faculty of Medicine, İstanbul, Turkey

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***Streptococcus pneumoniae* carriage is a risk factor for the development of respiratory system infections and the spread of penicillin-resistant strains.**

The aim of this study was to investigate nasopharyngeal carriage of *S. pneumoniae* in healthy children and resistance to penicillin and other antimicrobials and to assess related risk factors.

Nasopharyngeal specimens collected from healthy children less than six years of age, visiting a Mother and Child Health Center for health control, were investigated microbiologically between February-March 2004.

Carriage rate was 37.2% (n=112/301); 33.9% intermediate and 5.4% high penicillin resistance were detected. According to multivariate analysis, carriage rate was inversely related to number of rooms (OR:0.574) and child age (OR:0.978), while penicillin resistance was correlated well with antibiotic use in the last two months (OR:2.193). Decreased sensitivity plus resistance to other antimicrobials were: trimethoprim-sulfamethoxazole (TMP-SMX) 45.6%; erythromycin 16.1%, tetracycline 16.1%; clindamycin 9.8%, and ofloxacin 3.6% in pneumococcal isolates, which increased significantly ($p<0.05$) to 72.7%, 31.8%, 27.3%, 20.5%, and 6.8%, respectively, in penicillin non-sensitive *S. pneumoniae* (PNSSP) except for ofloxacin. Overall multidrug resistance was 17.9%, while PNSSP exhibited a resistance rate of 38.6%.

In conclusion, *S. pneumoniae* carriage rates determined in healthy children were high and PNSSP strains also showed increased resistance to other antimicrobials.

Key words: S. pneumoniae, carriage, penicillin resistance.

Streptococcus pneumoniae (*S. pneumoniae*) is an important pathogen that causes pneumonia, sinusitis, otitis media, and meningitis in children[1,2].

Children may carry *S. pneumoniae* asymptomatically and more commonly than adults[3]; colonization rates vary from 8.6 to 66.0% as reported in different studies[1,4-7]. Several risk factors such as day care center attendance[5], age <5 years, presence of a sibling with a positive culture, residence in a community[6], and one or more episodes of sinusitis in the previous three months[4] are associated with the nasopharyngeal colonization of *S. pneumoniae* among children. Nasopharyngeal carriage of *S. pneumoniae*, in the presence of several factors, such as preceding viral infections, bronchial obstruction, alteration of mucociliary function by allergy or irritants, splenectomy, human immunodeficiency virus (HIV) infection and other immune compromising conditions, predisposes to invasive pneumococcal infections[1,2].

S. pneumoniae is the most common cause of bacterial acute respiratory tract infections (ARI), which are the leading cause of mortality among children especially in developing countries[3].

In Turkey, ARI is still the most prevalent disease among children under five years of age especially during winter months[8].

Until the mid-1970's, pneumococcal infections were treated by relevant antibiotics; however, during the past two decades, pneumococci have increasingly become resistant to penicillin and other antibiotics due to bacterial genetic variations and to geographic spread, which were facilitated by frequent use of antibiotics[1]. The nasopharyngeal carriage of antibiotic-resistant pneumococci has been investigated in pediatric populations worldwide. Penicillin resistance rates reported from different countries varied from 9.1% to 37.1%[4,6,9-12]. Penicillin resistance was as high as 70.4%-91.3% in some Asian countries[9]. Results of different studies conducted in Turkey indicated an intermediate resistance of 24.3%-39.9% and a high resistance of 0%-3.9% to penicillin in clinical isolates[13-17]. *S. pneumoniae* carriage rate was reported as 28-43% among children with respiratory tract infection (RTI), bacteremia or bacterial meningitis[18,19] and as 8.5-30%[19,20] among healthy children.

The emergence of antibiotic resistance among pneumococci is the main cause for concern regarding treatment failure for pneumococcal infections. This concern led to the revision of management strategies to treat pneumococcal infections caused especially by intermediately resistant strains. This resulted in the use of higher dosages of penicillin for pneumococcal infections other than meningitis. The occurrence of invasive infections with resistant strains and concerns about treatment failure emphasize the need for knowledge about factors that affect nasopharyngeal carriage of resistant pneumococci. Young age, day care center (DCC) attendance and prior antibiotic treatment were previously shown to be associated with high carriage rate of antibiotic-resistant *S. pneumoniae*[4,9-12].

In Turkey, only a limited number of studies have been performed to determine nasopharyngeal colonization rate of penicillin-sensitive and penicillin-non-sensitive *S. pneumoniae* (PNSSP) in children and to investigate the risk factors for the colonization[19-21].

The aim of this study was to investigate the nasopharyngeal carriage rate of *S. pneumoniae* in healthy children and its resistance to penicillin and to other antimicrobials and to assess the risk factors for carriage.

Material and Methods

Setting

This prevalence study was performed between February 2 and March 5, 2004 in a Primary Care Center (Umraniye Mother and Child Health Center) in İstanbul, Turkey. The study was approved by the Marmara University Medical School Ethics Committee.

Data Collection

Cases

Nasopharyngeal specimens were obtained from 369 children ranging from 9 days to 67 months who visited the health center for a health control and/or immunization. Every healthy child younger than six years and whose parents had given informed consent was considered as eligible for inclusion. This age group was chosen due to high risk of pneumococcal infection.

Sample Size

We estimated that approximately 8.5% of children would carry *S. pneumoniae*. According to the sample size previously calculated using INSTAT analysis program for 95% confidence interval with 0.05 standard error, it was determined that a minimum of 289 participants was required.

Risk Factors for Nasopharyngeal Carriage of S. pneumoniae and PNSSP Carriage

Information on potential risk factors that may affect carriage was obtained from questionnaires administered to the parents by face-to-face interview. Data collected from the parents included: age and gender of the children, education, income and social security of their parents, number of persons living in the same house with the child, number of room(s) in the house, number of siblings, age of siblings, DCC attendance, antibiotic treatment in the past two months, hospitalization and acute otitis media (AOM) in the last year, the use of at least one cure of antibiotic by family members in the last three months and smoking habit of people in the household.

Screening and Microbiologic Methods

A single specimen per child was collected from nasopharynx with sterile cotton-tipped flexible swabs (LP Italiana) and all the specimens were

transferred by Stuart transport medium (Copan, Italia) to the Microbiology Laboratory of Marmara University Hospital within five hours. Sampling was performed by four students trained especially for this study on mannequins, in the Clinical Skills Laboratory of the Marmara University Medical School.

The swabs were streaked onto Columbia agar supplemented with 5% sheep blood (Biomérieux, France) and incubated at 35°C for 24 hours. After 24 hours, gram-positive cocci which were catalase-negative and alpha hemolytic colonies were tested for optochin sensitivity and bile solubility and identified as *S. pneumoniae*.

The sensitivity of *S. pneumoniae* to oxacillin (1 μg) was tested by the disk diffusion method on Mueller-Hinton agar plates supplemented with 5% sheep blood, according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS)[22]. All isolates suspected of being PNSSPs (oxacillin susceptibility <20 mm) were further tested for penicillin-G susceptibility by the E-test method (AB Biodisk, Sweden). PNSSPs were defined as isolates with a minimal inhibitory concentration (MIC) ≥2 μg/ml.

S. pneumoniae strains were tested for sensitivity to vancomycin, tetracycline, ofloxacin, clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX) and erythromycin by disk diffusion method. Standard NCCLS susceptibility breakpoints were used to classify organisms as susceptible, intermediate, and resistant.

Statistics

Sociodemographic and microbiologic data were statistically analyzed by chi-square test and logistic regression models in SPSS 11.5 software program. Potential risk factors were tested in separate bivariate analyses for their association with 1) nasopharyngeal carriage of *S. pneumoniae* and 2) carriage of PNSSP among children colonized with *S. pneumoniae*.

Univariate analysis of categorical variables was performed with chi-square or Fisher's exact test where appropriate. To further assess potential risk factors for colonization with *S. pneumoniae* or PNSSP, multivariate analysis was performed based on significant bivariate predictors and variables of particular interest based on previous studies, even when not statistically significant. A p value less than 0.05 was accepted as statistically significant. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using SPSS 11.5. Separate multivariate models including such predictors were constructed for carriage of *S. pneumoniae* and for carriage of PNSSP; non-significant variables were removed sequentially until only those significant at $p < 0.05$ remained.

Results

A total of 369 children were screened. Three hundred and one specimens were eligible and were evaluated statistically. The results of the remaining 68 samples were excluded since they were found to have a recent or current RTI during detailed physical examination. The characteristics of the children are summarized in Table I. None of the children was attending a DCC. *S. pneumoniae* was isolated from 112 children (carriage rate 37.2%); 44 of these isolates were PNSSP (39.3% of carriers and 14.6% of all children). Thirty-eight (33.9%) of the strains showed intermediate (MIC 0.12-1 μg/ml) and six (5.4%) showed high-level (MIC ≥2.0 μg/ml) resistance to penicillin.

Overall rates of resistance to other antimicrobials, defined as the rate of intermediate resistance plus the rate of resistance were as follows: TMP-SMX 45.6% (n=51), tetracycline 16.1% (n=18), erythromycin 16.1% (n=18), clindamycin 9.8% (n=11), and ofloxacin 3.6% (n=4). No resistance was observed to vancomycin (Table II). Cross-resistance between PNSSP and other antimicrobials is summarized in Table III. The rates of resistance to TMP-SMX, erythromycin, tetracycline and clindamycin showed statistically significant increase among PNSSP isolated species. Although resistance to tested quinolone was higher in PNSSP compared to susceptible ones, the increase was not significant. All strains, both PSSP and PNSSP, were fully susceptible to vancomycin. Twenty of 112 strains (17.9%) were multidrug resistant, which means resistance to three or more antimicrobials; 9 (8.0%) strains were resistant to 3 antimicrobials, 3 (2.7%) strains to 4 antimicrobials and 8 (7.1%) of them were resistant to 5 antimicrobials (penicillin, TMP-SMX, clindamycin, erythromycin and tetracycline). The cross-analysis indicated that multidrug resistant strains were mostly accumulated in younger age groups. Multidrug

Table I. Characteristics of Children Providing Nasopharyngeal Specimens

	Number	
Characteristics	n=301	(%)
Gender		
Female	141	46.8
Male	160	53.2
Age (month)		
Mean 13.63	Min: 9 days	Max: 5 y 8 mo 23 days (=67.77 mo)
0-3	45	14.9
>3-6	74	24.6
>6-9	31	10.3
>9-12	42	14.0
>12	109	36.2
Parental education		
Mother		
≤5 years	191	63.5
>5 years	110	36.5
Father		
≤5 years	138	45.8
>5 years	163	54.2
Income (monthly)		
≤600 NTL	193	64.1
>600 N TL	108	35.9
Social security		
Yes	216	71.8
No	85	28.2
Number of rooms	Mean: 3.34	Min: 1 Max: 5
1-3	158	60.19
≥3	120	39.9
Number of family members	Mean: 4.45	Min: 3 Max: 13
≤3	83	27.6
4	117	38.9
5	47	15.6
≥6	54	17.9
Number of siblings		
No sibling	117	38.9
1 sibling	121	40.2
>1 sibling	63	20.9
Respiratory tract disease in the last 3 months	169	4.7[1]
At least one attack of AOM in the last year	31	38.7[2]
Hospitalization in the last year	9	77.8[3]
Antibiotic use in the last 2 months	149	1.3[4]
Antibiotic use of the family members in the last 3 months		
Yes	130	43.2
No	171	56.8
Hospitalization of family members in the last 3 months	16	5.3
Smoking at home		
Yes	190	63.1
No	111	36.9

NTL: New Turkish lira ($1=1.350 NTL). AOM: Acute otitis media.
[1]Percentage was given for children aged 0-3 months (n=8/169) who had respiratory tract diseases in the last 3 months.[2] Percentage was given for 0-1 year of age children (n=12/31) who had at least 1 attack of AOM in the last year. [3]Percentage was given for 0-1 year of age children (n=7/9) who had been hospitalized in the last year. [4]Percentage was given for children aged 0-2 months (n=2/149) who had used antibiotic in the last 2 months.

Table II. Overall Resistance Rates of Isolated *S. pneumoniae* Strains to Antimicrobials

S. pneumoniae	*Sensitive*		Intermediate		Resistant	
Antimicrobials	n	%	n	%	n	%
Penicillin	68	60.7	38	33.9	6	5.4
TMP–SMX	61	54.4	5	4,5	46	41.1
Erythromycin	94	83.9	1	0.9	17	15.2
Tetracycline	94	83.9	–	–	18	16.1
Ofloxacin	108	96.4	2	1.8	2	1.8
Clindamycin	101	90.2	–	–	11	9.8
Vancomycin	112	100.0	–	–	–	–
Total	112 (100%)					

TMP-SMX: Trimethoprim-sulfamethoxazole.
*Penicillin sensitivity is determined by E-Test; ≤0.06 μg/ml is considered as sensitive, 0.12-1 μg/ml as intermediate and >1 μg/ml as resistant.

Table III. Cross-Resistance of PSSP and PNSSP to Other Antimicrobials

	Susceptibility levels								
	PSSP (n=68)				PNSSP (n=44)				
	Susceptible		Resistant*		Susceptible		Resistant*		
Antimicrobials	n	%	n	%	n	%	n	%	p[o]
TMP–SMX	49	72.1	19	27.9	12	27.3	32	72.7	0.000
Erythromycin	64	94.1	4	5.9	30	68.2	14	31.8	0.000[a]
Tetracycline	62	91.2	6	8.8	32	72.7	12	27.3	0.009
Clindamycin	66	97.1	2	2.9	35	79.5	9	20.5	0.006[a]
Ofloxacin	67	98.5	1	1.5	41	93.2	3	6.8	0.298[a]
Vancomycin	68	100	–	–	44	100	–	–	–

TMP-SMX: Trimethoprim-sulfamethoxazole. PSSP: Penicillin-sensitive *S. pneumoniae*.
PNSSP: Penicillin-non-sensitive *S. pneumoniae*.
Resistant* includes all intermediate and resistant strains.
[o]*Chi square test* $P<0.05$ is significant.
[a]*Fisher's exact test*.

resistant phenotypes and their distribution among PSSP and PNSSP are shown in Table IV. There were more multidrug resistant strains in the PNSSP group (n=17/44; 38.6%) than the PSSP group (n=3/68; 4.4%), which supports the results of Table III.

Risk Factors for Nasopharyngeal Carriage of S. pneumoniae and PNSSP

Number of rooms ≤3 was significantly related to the carriage of *S. pneumoniae* according to the univariate analysis of independent risk factors. Gender, age, parents' education, income, social security, number of family members, number of siblings, prematurity, RTI in the last three months, AOM in the last year, antibiotic use in the last two months, antibiotic use and hospitalization of family members in the last three months and smoking at home had no statistically significant relationship with carriage of *S. pneumoniae* by univariate analysis ($p>0.05$).

The results of the multivariate analysis in which parents' education, income, number of rooms, number of siblings and family members living in the house, age (month), antibiotic use in the last two months, having a sibling under two years and AOM in the last year were included revealed that young age (OR=0.978, 95% CI: 0.959-0.998) and number of rooms in the house (OR=0.574, 95% CI: 0.395-0.834) were significant variables for the risk of carrying *S. pneumoniae*. Antibiotic use in the last two months was the only risk factor for nasopharyngeal carriage of PNSSP according to both univariate and multivariate analysis results (Table V).

Table IV. Multidrug Resistant Phenotypes Among All *S. pneumoniae* (n=20)

	PSSP	PNSSP
Phenotypes	n	n
Erythromycin + Ofloxacin		1
TMP-SMX + Erythromycin		2
TMP-SMX + Ofloxacin		1
TMP-SMX + Tetracycline		2
Erythromycin + Tetracycline + Clindamycin	1	
TMP-SMX + Erythromycin + Clindamycin		1
TMP-SMX + Erythromycin + Tetracycline	2	2
TMP-SMX + Erythromycin + Tetracycline + Clindamycin		8
Total	3	17

TMP-SMX: Trimethoprim-sulfamethoxazole. PSSP: Penicillin-sensitive *S. pneumoniae*.
PNSSP: Penicillin-non-sensitive *S. pneumoniae*.

Table V. Results of Univariate and Multivariate Analysis for Carriage of S. pneumoniae and PNSSP

	S. pneumoniae			
	Univariate		Multivariate	
Risk factors	OR (95% CI)	p value	OR (95% CI)	p value
Number of rooms	0.556 0.340-0.909	0.019	0.574 0.395-0.834	0.004
Age (months)			0.978 0.959-0.998	0.028
	PNSSP			
	OR (95% CI)	p value	OR (95% CI)	p value
Antibiotic use in the last 2 months	2.193 1.013-4.750	0.045	2.193 1.013-4.750	0.046

TMP-SMX: Trimethoprim-sulfamethoxazole. PSSP: Penicillin-sensitive *S. pneumoniae*. PNSSP: Penicillin-non-sensitive *S. pneumoniae*. OR: Odds ratio. CI: Confidence interval.

Discussion

Colonization Rate and Contributing Risk Factors

Nasopharyngeal carriage rate for *S. pneumoniae* in our study was determined as 37.1%, similar to the result (30%) of another study performed by Çiftçi et al.[19] among children who had been admitted to a university hospital in Ankara, Turkey. The carriage rate of *S. pneumoniae* in the pharyngeal specimens of healthy children was determined as 8.5% in Bakır et al.'s[20] epidemiological study which had been realized in a total of 24 centers including healthy child outpatient clinics, DCCs and primary care schools in İstanbul. This rate was lower than the results of the two former studies, which were conducted solely in health care centers and in which the specimens had been collected from the nasopharynx. These two main differences could explain the higher level of carriage rate. The colonization rate determined in our study was higher than that reported from Italy (8.6%)[4] and the United States (14-24%)[6] and lower than the rates reported from Sweden (52%)[5] and Russia (44.9-66.0%)[7]. It is well documented that nasopharyngeal carriage of *S. pneumoniae* is a predisposing factor for pneumococcal infections such as otitis media, mastoiditis, sinusitis, and meningitis. Since lower RTIs are still among the leading causes of death in children under five years of age in our country[8], this high rate (37.1%) of nasopharyngeal carriage can be a potential threat for this age group.

In the literature, colonization rates were independently related to risk factors such as one or more episodes of sinusitis in the previous three months[4], DCC attendance[5,23], age <5 years, presence of a sibling with a positive

culture, intrafamilial transmission, residence in a community[6], age ≤3 years, and living in a rural area[23]. Young age and number of rooms were determined as the contributing factors to nasopharyngeal carriage of *S. pneumoniae* in our study. None of the children in the study group was attending a DCC, but their houses had a limited number of rooms, which appeared as a risk factor in our study. Although it was not a statistically significant risk factor, number of family members in those houses was relatively high. Supporting our findings, Samore et al.[6] described intrafamilial transmission as an important risk factor for pneumococcal carriage.

Penicillin Resistance and Contributing Risk Factors

Antimicrobial resistance is a growing problem among respiratory tract pathogens. Nasopharyngeal colonization by antibiotic-resistant *S. pneumoniae* has steadily increased over the last years[1,4]. Carriage rate of resistant strains was found as 39.3% in our study population, with an intermediate resistance of 33.9% and high resistance of 5.4%. These results seems to discourage physicians in using penicillin in empirical treatment of pneumococcal infections, particularly in the case of meningitis, where intermediately resistant strains responsible for the infection would not respond even to high dosages of penicillin. Supporting our findings and our comments, Çiftçi et al.[19] also found intermediate resistance rate as 32.7%.

High carriage rate of PNSSP was previously shown to be associated with young age, DCC attendance, having young siblings, having an upper RTI such as AOM (recently or during the study), recent antibiotic treatment and residence in urban areas[4,9-12]. Similar factors were determined in two studies performed in our country[18,21]. In our study, the only risk factor for carriage of penicillin-resistant *S. pneumoniae* was at least one antibiotic cure in the last two months. In a study from Sweden, previous antibiotic use and DCC attendance were not determined as risk factors for carriage of PNSSP; the authors suggested that young children may acquire resistant strains from their sibling (intrafamilial transmission), friends or family members[6]. Although there was no child attending DCC in our study group, carriage rate of PNSSP was high but not significantly related to the number of siblings and family members.

Resistance to Other Antibiotics and Cross-Resistance

In our study, the highest antibiotic resistance was against TMP-SMX; resistance to tetracycline, erythromycin and clindamycin were 16.1%, 15.2%, and 9.8%, respectively. Other studies performed in our country reported similar reduced susceptibility rates to tetracycline (21.8-31%), erythromycin (8-19%), and TMP-SMX (31.5-55.4%)[13,15,18,19]. TMP-SMX was also the least active antimicrobial with high resistance rates, between 28% to 53.4%, as determined by different authors from the United States[6,24], Canada[10] and Russia[7]. These results implicate the restriction of TMP-SMX use in the treatment of pneumococcal infections. In these studies, resistance rates to other antibiotics reported as 13.0%[10]-18.8%[11] for erythromycin, 7.0[10] and 12.6%[11] for clindamycin, and 17.1%[11] for tetracycline were also similar to those of our study. Macrolide resistance rates were lower than those reported from Italy (52.1%)[4] and Spain (34.5%)[25]. In the report of PneumoWorld Study (2001-2003) overall, 28% of *S. pneumoniae* isolates were resistant to macrolides. The prevalence of resistance varied among European countries, with the highest rates reported from Spain and France[26]. In some Asian countries such as Korea, Vietnam, and China, macrolide resistance is also very high[9]. As safe and well-tolerated antibiotics, macrolides play an important role in the treatment of RTIs. Therefore, the possibility of increase in resistance to these antimicrobials may lead to the restriction of their clinical usage.

In our study, susceptibility of the strains to the other antibiotics decreased significantly in the presence of penicillin resistance. PNSSP had decreased sensitivity plus resistance to TMP-SMX (72.7%), erythromycin (31.8%), tetracycline (27.3%), and clindamycin (20.5%). Bakır et al.[20] reported cross-resistance as follows: TMP-SMX 85%, tetracycline 62%, and erythromycin 18%, and 68% of the PNSSP isolates were resistant to two or more different classes of antibiotics in addition to penicillin. In the Asian multinational study, penicillin resistant isolates were also highly resistant to TMP-SMX (88.5%), tetracycline (91.2%), and erythromycin (95.1%)[9]. The authors suggested that penicillin and multidrug resistance was a serious problem in many countries in Asia and the Middle East due to the spread of several

predominant drug-resistant clones. Similarly in Greece, 50% of PNSSP were also resistant to erythromycin, whereas total prevalence of erythromycin resistance was 19%[11]. In the same study, 64% of PNSSP were multidrug resistant and this rate was higher than that of PSSP (37%). Multidrug resistance rates were reported as 20-22.2% in two different studies from the United States[24,27]. In our study, this rate was 17.9% of *S. pneumoniae* isolates; however, it increased to 38.6% in penicillin-resistant strains. Our result was relatively low with respect to the rates reported by the other studies.

In conclusion, according to the results of our study, nasopharyngeal carriage of *S. pneumoniae* was high and inversely related to the age of the children and the number of rooms in the houses, indicating that physicians in this country should be aware of the risk for development of invasive pneumococcal infections at least in one-third of this age group. Furthermore, occurrence of such infections would carry the risk of treatment failure due to the high colonization rate of resistant strains. However, there is a need for a nationwide surveillance to provide data about the real dimensions of antibiotic resistance in our country.

The carriage rate for PNSSP was related to recent antibiotic use. Frequent use of antibiotics is known as the most important factor for the spread of resistance. To decrease the emergence and the spread of resistant strains, efforts were being made worldwide to reduce antibiotic use[3] since a significant relationship exists between penicillin resistance and the resistance to other antibiotics. In our country, the Ministry of Health has put in service guidelines for rational antibiotic use in the treatment of RTIs. However, frequent and unrestricted use of broad spectrum antibiotics continues[28] and antibiotics are still the most frequently used medications. The results of this study should alert physicians to follow guidelines while prescribing antibiotics.

Limitations of the Study

Our study was realized in a health center in İstanbul. Although the number of participants provided sufficient data to perform a statistical analysis, the study did not have an epidemiological design to represent all the children in İstanbul.

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