Antimicrobial susceptibility of bacterial pathogens in the oropharynx of healthy school children in Turkey

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Background & objectives: Information on oropharyngeal carriage rates of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes* and *Moraxella catarrhalis* and their resistance pattern in healthy school children in Turkey is lacking. The present study was undertaken to determine the carriage rates and antimicrobial resistance of these bacterial pathogens in such children aged 6-14 yr in Manisa, Turkey.

Methods: A total of 1022 children were included from nine schools selected randomly from 32 schools. Throat swabs were cultured for bacteria which were identified using standard microbiological methods. Antimicrobial susceptibility was determined as per National Committee for Clinical Laboratory Standards guidelines.

Results: Of the 1022 children 240 (23.4%) harboured *S. pneumoniae*, 162 (15.8%) *H. influenzae*, 30 (2.9%) *S. pyogenes* and 82 (8%) *M. catarrhalis* in their oropharynx. For *S. pneumoniae* overall 17.9 per cent of the isolates were intermediately and 7 per cent were resistant to penicillin and resistance to erythromycin trimethoprim-sulphamethoxasole (TMP/SMX), and chloramphenicol was 13.7, 9.1 and 1.6 per cent, respectively. Ampicillin resistance observed in 20.9 per cent of *H. influenzae* isolates was associated with the presence of β-lactamase, except two isolates interpreted as β-lactamase-negative ampicillin resistant strains. Resistance of *H. influenzae* to TMP/SMX, chloramphenicol, azithromycin, ceftacor and amoxicillin/clavulanic acid was 14.2, 2.4, 1.8, 1.2 and 1.2 per cent, respectively. *M. catarrhalis* isolates produced β-lactamase in 80.5 per cent of the cases and all were susceptible to macrolides and clavulanic acid/amoxicillin combination; the highest rate of resistance of 17 per cent was for TMP/SMX. One (3.3%) isolate of *S. pyogenes* was resistant to macrolides tested.

Interpretation & conclusion: Our data shows that upper respiratory tract of about 50 per cent children was colonized with respiratory pathogens. There is a need for surveillance of nasopharyngeal carriage of resistant strains in healthy school children.

Key words Antimicrobial resistance - oropharyngeal carriage - school children

The upper respiratory tract of healthy children is frequently colonized with *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. This nasopharyngeal colonization is a prerequisite for the development of sinusitis, otitis media or more serious infections. Emergence of resistant bacteria such as β-lactamase producing *M. catarrhalis* and *H. influenzae*, penicillin resistant *S. pneumoniae* and macrolide resistant *S. pyogenes*, has complicated the empirical therapy of these infections in several countries.
In general, the prevalence of carriage of these pathogenic bacteria in healthy individuals decreases with age. For this reason, epidemiologic studies on the nasopharyngeal carriage of these pathogens have been performed on children under 6 yr of age. There is lack of information on the carriage rates and resistance patterns of these pathogens in Turkish school children. As surveillance of nasopharyngeal carriage of resistant strains is important for initiating adequate empirical antimicrobial therapy, the present study was carried out to determine the carriage rates and antimicrobial resistance patterns of S. pneumoniae, H. influenzae, S. pyogenes and M. catarrhalis isolated from healthy school children in Manisa, Turkey.

Material & Methods

A cross sectional study was conducted in children 6-14 yr of age from November 2002 to January 2003. Relevant information on the study population was obtained from the Bureau of Statistics of the Provincial Education Administration of Manisa city, west Turkey. Information revealed that there were 32 schools in the Manisa city center with a total of 32,244 students enrolled. We stratified the schools into 3 strata according to the average socio-economic status, i.e., low, moderate and high. Three schools were randomly selected from each stratum. The sample size was estimated as 1000 students with a confidence level of 95 per cent, prevalence of carriers of pathogens=30 per cent, s=0.03. The number of students in selected schools was 12,475. Each school had eight grades with approximately 40 students in each class. Equal number of students was chosen randomly from each grade and the number of students chosen from each school was proportional to the student population of each school. As a result a total of 1,022 children were included in the study. Informed consents from the parents, and approval from the ethical committee of the institution have been obtained prior to the commencement of the study. Personal information about those participated in the study was obtained using standardized questionnaires for sociodemographic variables. All children underwent a physical examination regarding upper respiratory tract infection. Children with no signs of respiratory infection and history of receiving antibiotic treatment within the past 2 wk were included in the study.

Throat swabs taken from tonsils and anterior pillars of the oropharynx were immediately introduced in Stuart transport medium (Oxoid Limited, Hampshire, England) and sent to the laboratory within 2 h of collection. Specimens were seeded on sheep blood agar (Oxoid Limited, Hampshire, England) for isolation of S. pneumoniae and S. pyogenes, and on chocolate agar prepared by adding sheep blood to an enriched agar base medium (Oxoid Limited, Hampshire, England) at 80°C for M. catarrhalis and H. influenzae. After 24-48 h incubation in a CO₂ enriched atmosphere at 37°C, plates were inspected for growth of bacteria. All isolates were identified by the standard microbiological methods and were confirmed as S. pneumoniae, H. influenzae, S. pyogenes and M. catarrhalis on the basis of commercial kits (BBL GP ID; Crystal N/H ID, Becton Dickinson and Co., Sparks, MD, USA). The capsular antigenic typing of H. influenzae was done by slide co-agglutination with commercial antisera (Difco, Detroit, MI, USA).

Antimicrobial susceptibility by the disk diffusion method was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. The following antibiotics were used: azithromycin (15 µg) for all 4 bacteria; erythromycin (15 µg) for all except H. influenzae; trimethoprim-sulphamethoxasole (1.25/23.75 µg) and chloramphenicol (30 µg) for all except S. pyogenes; amoxicillin/clavulanic acid (20/10 µg) for H. influenzae and M. catarrhalis; ampicillin (10 µg) for S. pyogenes and H. influenzae; oxacillin (1 µg) for S. pneumoniae; and cefator (30 µg) for H. influenzae. Minimum inhibitory concentrations (MIC) of penicillin against S. pneumoniae isolates were determined by E test (AB Biodisk, Solna, Sweden) with an inoculum recommended by NCCLS for the disk diffusion method. Susceptibility to penicillin was categorized as susceptible if the MIC was < 0.1 µg/ml, intermediate between 0.1 and 1.0 µg/ml, and resistant if the MIC was > 1.0 µg/ml. The susceptibility tests were performed on Mueller-Hinton agar (Oxoid Limited, Hampshire, England) with 5 per cent sheep blood for S. pneumoniae and S. pyogenes, on Mueller-Hinton agar for M. catarrhalis, and on Haemophilus Test Medium (Oxoid Limited, Hampshire, England) for H. influenzae strains. All isolates of H. influenzae and M. catarrhalis were
examined for production of beta-lactamase with a nitrocefin-based test (Oxoid Limited, Hampshire, England). *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247 (Oxoid Limited, Hampshire, England) were used as quality control organisms for susceptibility tests.

A statistical package SPSS10.0 (SPSS incorporated, Chicago) was used for all analysis. A chi square test was used for comparison of categorical variables. A *P* value of <0.05 was considered statistically significant.

**Results**

Of the total 1022 children, *S. pneumoniae*, *H. influenzae*, *S. pyogenes* and *M. catarrhalis* were detected in 240 (23.4%), 162 (15.8%), 30 (2.9%) and 82 (8%) children, respectively. Of the 162 isolates of *H. influenzae*, 8 (4.9%) belonged to serotype b. No statistically significant difference was found between the socio-economic status and the carriage rate.

Of the 240 *S. pneumoniae* isolates investigated, 60 (25%) were found to be resistant to penicillin. Seventeen (7%) isolates were penicillin resistant (MIC≥1 µg/ml) and 43 (17.9%) were intermediately resistant (0.1 ≤ MIC ≤ 1 µg/ml). The MICs did not exceed 2 µg/ml for any of the isolates tested. Second to penicillin, the highest rate of resistance was against erythromycin (13.7%), followed by TMP/SMX (9.1%). Resistance to azithromycin was observed in 33 isolates, which were also resistant to erythromycin (13.7%). Resistance to chloramphenicol occurred in 1.6 per cent of the isolates. Among the 60 isolates that had intermediate or resistance to penicillin, 23 (38.3%) were also resistant to erythromycin and 16 (26.6%) to TMP/SMX. One isolate was fully resistant to penicillin, erythromycin, TMP/SXT and chloramphenicol.

Ampicillin resistance observed in 34 (20.9%) of 162 *H. influenzae* isolates was associated with the presence of beta-lactamase (19.7%), except in two which did not react with nitrocefin and were identified as β-lactamase negative ampicillin resistant (BLNAR) strains. These 2 (1.2%) isolates were also resistant to cefaclor and clavulanic acid/amoxicillin combination. Of the 34 isolates resistant to ampicillin, 8 (23.5%) were also resistant to cefaclor and clavulanic acid/amoxicillin combination. Of the 34 isolates resistant to ampicillin, 8 (23.5%) were also resistant to cefaclor and clavulanic acid/amoxicillin combination. Of the 34 isolates resistant to TMP/SMX, 14 (17%) isolates were resistant to TMP/SMX and 2 (2.4%) to chloramphenicol. Nevertheless all these isolates were susceptible to azithromycin (Table).

Of the 82 *M. catarrhalis* isolates, 66 (80.5%) were β-lactamase-positive. As there is no breakpoint given for antimicrobials in the NCCLS table that cover *Moraxella*, results were interpreted according to the criteria for non-fastidious bacteria. According to the breakpoints for *Staphylococcus* spp, all the isolates were susceptible to amoxicillin/clavulanic acid, erythromycin and azithromycin but 14 (17%) isolates were resistant to TMP/SMX and 2 (2.4%) to chloramphenicol.

None of the 30 *S. pyogenes* isolates tested were resistant to ampicillin, but only one isolate showed cross-resistance to erythromycin and azithromycin (3.3%).

**Discussion**

<table>
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<tr>
<th>Table. Percentage of <em>S. pneumoniae</em> and <em>H. influenzae</em> isolates resistant to antimicrobial agents</th>
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<tr>
<td><strong>Isolate</strong> (n)</td>
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<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td><em>S. pneumoniae</em> (240)</td>
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<tr>
<td><em>H. influenzae</em> (162)</td>
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</table>

*Resistance including resistant and intermediately resistant *S. pneumoniae* isolates

NT, not tested

Values in parentheses are number of isolates
We found that the upper respiratory tract of about 50 per cent of 6-14 yr old children was colonized with potential respiratory pathogens. Similar colonization rates for \textit{S. pneumoniae}, \textit{H. influenzae}, and \textit{M. catharralis} in comparable age group were found in some other studies\textsuperscript{6,13,14}.

Penicillin resistant \textit{S. pneumoniae} isolates from asymptomatic school children accounted for 25 per cent of the 240 isolates investigated in this study. Forty three isolates (17.9\%) were intermediately resistant. Although 17 strains (7\%) were resistant, surprisingly, the MICs did not exceed 2 mg/ml for any of isolates tested. In our country, comprehensive epidemiological studies on the carriage rate of \textit{S. pneumoniae} and its antibiotic resistance in prepubertal ages are not available. To the best of our knowledge, majority of the studies\textsuperscript{15,16} deals with antibiotic resistance of the strains isolated from children and adults with clinical diseases. In a multicentre study conducted by Gür \textit{et al}\textsuperscript{17}, including 283 \textit{S. pneumoniae} isolates from adults and children with clinical diseases, intermediate penicillin resistance was found to be 25.8 per cent and high resistance as 3.9 per cent. Sener \textit{et al}\textsuperscript{18} found intermediate penicillin resistance to be 39.9 per cent and high resistance to be 3.5 per cent with the agar dilution method on 143 \textit{S. pneumoniae} isolates from paediatric patients in 1993-96. These resistance rates were less than those calculated in recent studies carried out in Far East where 50-80 per cent of isolates were found to be highly resistant to penicillin\textsuperscript{19}. Strains with reduced susceptibility to penicillin are usually cross-resistant to other antibiotics\textsuperscript{19}. Previous studies from our country\textsuperscript{17,18} showed that the predominant antibiotic resistance pattern among pneumococci is penicillin-macrolides resistance. Similarly we found that 38.3 per cent of penicillin resistant isolates were also resistant to erythromycin and azithromycin. Resistance to erythromycin probably resulted from the frequent use of macrolides for the empirical treatment of upper respiratory tract infections in children in our country.

In the present study, 34 of the 162 \textit{H. influenzae} isolates had resistance to ampicillin (20.9\%). The incidence of \(\beta\)-lactamase producing isolates (19.7\%) was similar to that previously reported in other studies of \textit{H. influenzae} in Europe\textsuperscript{20}. In the USA, the frequency of \(\beta\)-lactamase production in \textit{H. influenzae} has been reported to be as high as 31.1 per cent\textsuperscript{21}. The BLNAR isolates were also observed in our study, but have rarely been mentioned in reports from our country\textsuperscript{17}. In Turkish children, Yagci \textit{et al}\textsuperscript{22} reported that the rate of \(\beta\)-lactamase production was 1-6.1 per cent and that BLNAR had not been observed. Another multicenter study\textsuperscript{17} reported 7 per cent \(\beta\)-lactamase production and 3.3 per cent of BLNAR in 272 \textit{H. influenzae} strains mostly isolated from patients with respiratory tract infection. Our isolates depicted a low resistance to chloramphenicol and azithromycin but more resistance to TMP/SMX. These resistance rates are similar to those reported from Turkey\textsuperscript{17,22}.

The production of \(\beta\)-lactamase among \textit{M. catarrhalis} was frequent and similar to that found in other studies\textsuperscript{5,21,23}. Among other antimicrobial agents, macrolides and amoxicillin/clavulonate were uniformly effective, \textit{i.e.}, all strains were susceptible whereas only 83 per cent were susceptible to TMP/SXT.

Macrolide resistant \textit{S. pyogenes} isolates are increasing in some parts of the world and 20-30 per cent resistance is being reported in some countries\textsuperscript{24}. The low rate of macrolides resistance in our isolates is quite similar to that recently reported from Turkey\textsuperscript{25}, indicating that macrolide resistant \textit{S. pyogenes} are rare in our country.

In conclusion, our results showed that nasopharyngeal carriage of potential respiratory pathogens in school children should not be underestimated. For this reason more comprehensive surveillance studies should be performed to obtain correct information about the carriage rate and antimicrobial resistance of these pathogens in Turkish children.

References


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