Risk factors for primary *Helicobacter pylori* resistance in Bulgarian children

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Risk factors for primary *Helicobacter pylori* resistance in 186 children with gastroduodenal diseases (44 from villages/small towns and 130 from large towns/cities) in 2000–2003 were tested. Susceptibility was tested by a limited agar dilution method. Overall resistance rates to metronidazole, clarithromycin, tetracycline and both metronidazole and clarithromycin were 14·5, 11·9, 3·3 and 4·3%, respectively. No amoxicillin resistance was observed. Tetracycline resistance was found in six children aged 7–18 years. Clarithromycin resistance was more common in children from small towns/villages (22·7%) than in those from large towns/cities (8·5%, *P* < 0·05). There were no significant differences (*P* > 0·05) in resistance rates between children from northern Bulgaria and those from southern regions. Resistance rates in duodenal ulcer patients and other children were, respectively, 10·5 and 15% (*P* > 0·20) for metronidazole and 10·5 and 12% (*P* > 0·20) for clarithromycin. No combined resistance to metronidazole and clarithromycin was found in 22 children aged 1–7 years and in 34 children living in northern Bulgaria. There were no significant associations of resistance with sex and age group (1–7–versus 8–18-year-old children) for all antibacterial agents tested. In conclusion, primary *H. pylori* resistance was absent (for metronidazole + clarithromycin) or low (4·5% for clarithromycin) in children aged 1–7 years. Place of residence was associated with clarithromycin resistance rates.

Introduction

Primary *Helicobacter pylori* resistance to antibacterial agents is one of the most important reasons for eradication failure, however, resistance rates may vary between groups of patients according to age, sex, disease and place of residence (Meyer et al., 2002; Fraser et al., 1999; Osato et al., 2001). Only few studies have evaluated the risk factors for primary *H. pylori* resistance in paediatric patients (Rerkasophaphol et al., 2003). The aim of the present study was to assess age, sex, disease and place of residence as possible risk factors for primary *H. pylori* resistance among Bulgarian children in 2000–2003.

Methods

*H. pylori* strains were isolated from 186 consecutive *H. pylori*-positive children, following routine endoscopy during investigation of upper gastrointestinal symptoms. Patients involved 84 boys and 102 girls, with chronic gastritis (149 cases), duodenal ulcer (19) and other conditions (18). Twenty-two children aged 1–7 years and 164 patients aged 8–18 years were evaluated. Of the children, 44 were from villages/small towns and 130 were from large towns/cities, 34 patients were from northern Bulgaria (in the north of the Balkan Range) and 140 children were from southern regions. Location data were not available for 12 children. The parents of patients signed written informed consents prior to endoscopy.

A single antral biopsy specimen was taken from each child and was transported in Stuart transport medium (Merck) for less than 5 h. A smear was used for a modified Gram stain with carbol fuchsin as the counterstain. A part of the biopsy specimen was placed in 10% urea agar medium (Boyanova et al., 1996). The urease test was incubated at 37°C and was read after 30 min and 3 h. The remaining part of the biopsy specimen was homogenized in 0·1 ml sterile saline and inoculated onto blood agar (Columbia agar base; Becton Dickinson) with *H. pylori* selective supplement, containing 10 μg vancomycin, 5 μg trimethoprim, 5 μg cefsulodin and 5 μg amphotericin B ml⁻¹ and/or 10% defibrinated sheep blood and 1% Isovitalex (Becton Dickinson). One selective and one non-selective medium plate were used for the primary culture of biopsy specimens. Plates were incubated for 3–12 days in a microaerophilic atmosphere.

*H. pylori* identification was made by Gram staining of the suspect colonies, lack of aerobic growth on blood agar plates and testing for the presence of urease, oxidase and catalase. The specimens were considered as *H. pylori* positive if only the culture or two of the three diagnostic methods were positive.

Susceptibility testing was performed by limited agar dilution method (Megraud et al., 1999; NCCLS, 2000). Two drops (approx. 60 μl) of *H. pylori* suspensions, prepared in Mueller–Hinton broth (National
Centre of Infectious and Parasitic Diseases, NCIPD) to obtain McFarland turbidity standard 3–4, were inoculated on a quarter of the surface of Mueller–Hinton blood agar plates (NCIPD) containing 1% Isovitalex and one of the following drug concentrations: 8, 16 and 32 μg metronidazole ml⁻¹, 0.25, 0.5, 1 and 2 μg clarithromycin ml⁻¹, 0.5, 1 and 2 μg amoxicillin ml⁻¹ and 4 μg tetracycline ml⁻¹. Antimicrobial agents were obtained from Sigma (amoxicillin, metronidazole and tetracycline) and Abbott Laboratories (clarithromycin). The plates were incubated microaerobically at 37°C for 3 days. If H. pylori growth appeared on the plate, the isolate was considered as resistant to the corresponding drug concentration. Non-selective medium plates were used as a control of strain viability.

The cut-off concentrations used to define resistance were: >8 μg metronidazole ml⁻¹, >1 μg clarithromycin ml⁻¹, >0.5 μg amoxicillin ml⁻¹ and >4 μg tetracycline ml⁻¹ (Megraud et al., 1999; NCCLS, 2000). Strains with MICs of 0.5 and 1 μg clarithromycin ml⁻¹ were considered as intermediately susceptible to clarithromycin.

Primary resistance rates in children were compared with those in 392 consecutive H. pylori-positive adult patients in 2000–2003. Differences between patients with susceptible and resistant strains were assessed by Chi-squared test with Yates’s correction.

### Results and Discussion

Overall primary resistance rates to metronidazole, clarithromycin, tetracycline and both metronidazole and clarithromycin in children were 14.5, 11.9 (16.7 for both intermediate susceptibility and resistance), 3.3 and 4.3 % (Table 1) and those in adults were 28.8, 11.7, 5.2 and 4.6 %, respectively (L. Boyanova, unpublished results). H. pylori resistance to metronidazole was more common in adults than in children. There were no statistically significant differences in primary resistance rates between children and adult patients (P > 0.10) except for metronidazole (P < 0.001). Amoxicillin resistance was observed in 1 % of H. pylori strains from adults but was not detected in children.

Clarithromycin resistance rate in Bulgarian paediatric patients was only significantly associated with place of residence (living in small towns or villages). The differences may be associated with local usage of macrolides. Clarithromycin resistance was significantly higher in children from small towns/villages (22.7 %) than in those from large towns/cities (8.5 %, P < 0.05; Fig. 1). Clarithromycin resistance (30.4 %) was more prevalent in children from small towns than in those living elsewhere (9.3 %, P < 0.02). Rates of both clarithromycin resistance and intermediate susceptibility were 10.5 % in duodenal ulcer patients and 17.5 % (P > 0.20) in other children (Fig. 2).

According to several studies, clarithromycin resistance in adults has been associated with geographical region, older age, female gender and non-ulcer diseases, although no

### Table 1. Primary H. pylori resistance to antimicrobial agents according to age group and sex in 186 Bulgarian children

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>Group of patients (age in years)</th>
<th>No. patients</th>
<th>% resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>1–7</td>
<td>22</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>8–18</td>
<td>164</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>84</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>102</td>
<td>15.7</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>1–7</td>
<td>22</td>
<td>4.5 (9.1*)</td>
</tr>
<tr>
<td></td>
<td>8–18</td>
<td>163</td>
<td>12.9 (17.8*)</td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>83</td>
<td>10.8 (14.4*)</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>102</td>
<td>12.7 (18.6*)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1–7</td>
<td>22</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>8–18</td>
<td>161</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>83</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Metronidazole + clarithromycin</td>
<td>1–7</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8–18</td>
<td>163</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>83</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>102</td>
<td>5.9</td>
</tr>
</tbody>
</table>

*Strains with both resistance and intermediate susceptibility to clarithromycin.
significant association of the resistance has been detected with age, sex, place of residence and ethnicity by other authors (Meyer et al., 2002; Fraser et al., 1999; Osato et al., 2001). In the present study, the primary clarithromycin resistance rate in Bulgarian children was lower than those in Spanish (28.3%), French (21%) and Polish (23.5%) paediatric patients (Dzierzanowska-Fangrat et al., 2001; Lopez-Brea et al., 2001) but was similar to that in Belgian children (16.6%) (Bontems et al., 2001). Although primary H. pylori resistance to newer macrolides has been more prevalent in children than in adults (Cabrita et al., 2000), in both groups of Bulgarian patients, the rate of clarithromycin resistance was about 12%.

Resistance to nitroimidazoles was detected even in the youngest patients. Two of nine strains isolated from children aged 1–4 years were metronidazole-resistant. Prevalence of metronidazole resistance in Bulgarian children was lower than that in Spanish and French paediatric patients (about 43%) and similar to that in Belgian children (18%) (Kalach et al., 2001; Bontems et al., 2001; Lopez-Brea et al., 2001).

Metronidazole resistance rates were 10.5% in children with duodenal ulcers and 15.0% (P > 0.20) in other paediatric patients. Metronidazole resistance was found in 19.6% of children living in the capital city Sofia and in 9.1% (0.1 > P > 0.05) of those living elsewhere.

According to the literature, metronidazole resistance in adults has been significantly associated with female gender, older age and ethnicity (Parsons et al., 2001; Fraser et al., 1999; Meyer et al., 2002; Pilotto et al., 2000; Katelaris et al., 1998). In adulthood, female gender has been a significant risk factor for antibiotic resistance in H. pylori (Pilotto et al., 2000), whereas in the present study, there were no significant associations of resistance (P > 0.10) with sex and age groups (1–7 versus 8–18 years of age) for all antibacterial agents tested.

Combined resistance to metronidazole and clarithromycin was found in 4.9% of the paediatric patients aged 8–18 years but was not detected in younger children.

No tetracycline resistance was detected in patients aged 1–6 years, probably because the use of tetracyclines in young children is inadvisable. In contrast to other studies (Samra et al., 2002; Cabrita et al., 2000), tetracycline resistance was found in six (3.3%) of 183 children, involving three (3.1%) of 97 patients living in the capital Sofia. The youngest patient harbouring a tetracycline-resistant H. pylori strain was a 7-year-old boy. Although rare, the emergence of tetracycline resistance in H. pylori strains from Bulgarian children is worrying.

There were non-significant differences (P > 0.05) in primary resistance rates between children living in northern Bulgaria and those living in southern regions: 2.9 versus 17.8% for metronidazole, 8.8 versus 12.8% for clarithromycin, 3.6 versus 2.8% for tetracycline and 0 versus 5.0% for both metronidazole and clarithromycin. In different regions of Canada, rates of H. pylori resistance to metronidazole and clarithromycin have been found to vary from 11 to 48% and from 0 to 12%, respectively (Fallone, 2000). According to a recent European multicentre survey, the resistance of H. pylori to metronidazole and clarithromycin in southern European countries has been higher than in other countries (Glupczynski et al., 2001). In the present study, no combined resistance to metronidazole and clarithromycin was detected in 34 children living in northern Bulgaria and the prevalence of metronidazole resistance in this group of patients was low (2.9 versus 17.8%) in children living in southern areas, 0.10 > P > 0.05).

In conclusion, primary H. pylori resistance was absent (for metronidazole + clarithromycin) or low (for clarithromycin) in patients aged 1–7 years. Place of residence was significantly associated with clarithromycin resistance rate and probably has a slight influence on the prevalence of metronidazole resistance. The high rate of primary clarithromycin resistance in children living in small towns or villages is surprising and is worth further evaluation. The risk factors for primary H. pylori resistance in children should be considered by clinicians in order to choose appropriate antibacterial agents for eradicating H. pylori infection or to request culture and susceptibility testing, when possible.

References


Dzierzanowska-Fangrat, K., Rozynek, E., Jozwiak, P., Celinska-


