High Level of Resistance to Metronidazole and Clarithromycin in *Helicobacter pylori* Isolated from Pediatric Patients in Poland (1997–2001)

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ABSTRACT. Resistance to metronidazole (Met), clarithromycin (Cla) and amoxycillin (Amo) was examined using *H. pylori* isolates from child patients before and after treatment in the period 1997–2001. The rate of Met and Cla resistance before treatment was 35.2 and 8.6 %, respectively. Six weeks after treatment 48.4 % of the isolated strains were resistant to Met and 17.6 % to Cla. The highest rate of resistance to both antibiotics was determined in 2001 (before treatment, 46 and 15 %, respectively, and after treatment, 57.8 and 26.3 %, respectively). All the strains were susceptible to Amo. Strains resistant to Met were detected more frequently in girls than in boys.

Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>Amo</td>
<td>amoxycillin</td>
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<td>Cla</td>
<td>clarithromycin</td>
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<td>Met</td>
<td>metronidazole</td>
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<td>Ome</td>
<td>omeprazole</td>
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Infection by *Helicobacter pylori* is the major cause of many diseases of the gastrointestinal tract, e.g., chronic gastritis, peptic ulcer and gastric cancer. Although many antibiotics exhibit activity against *H. pylori in vitro*, therapy with a single antibiotic is usually ineffective in clinical practice. Successful treatment of *H. pylori* infection most often requires the administration of two or more antibiotics (Met, Cla, Amo or tetracycline) and the addition of either a bismuth preparation or a proton pump inhibitor (Glupczynski et al. 1992; Vaira et al. 2002). It is therefore crucial for treatment efficacy to investigate the local antibiotic resistance of *H. pylori*, before the infecting strains are resistant, successful eradication is compromised.

We examined the susceptibility of *H. pylori* strains (isolated from infected children during 1997–2001) to Met, Cla and Amo.

MATERIALS AND METHODS

*H. pylori* strains. A total of 539 strains were isolated from gastric biopsy specimens (409 before treatment and 130 after treatment) of children aged 6–18 years with gastric or duodenal disorders. The patients were diagnosed and treated in the Second Clinic of Pediatrics, Gastroenterology and Nutrition, Medical University of Wrocław (Poland) in 1997–2001. None of the infected children was treated for *H. pylori* with antibiotics prior to isolation. The isolated strains were identified as *H. pylori* by Gram staining, morphology, as well as by positive urease, oxidase and catalase tests.

Susceptibility of strains to antibiotics (Met, Cla, Amo) was determined by the E-test (AB-Biodisk, Sweden) on Mueller–Hinton agar (Oxoid, UK) supplemented with 7 % horse blood under microaerophilic conditions. Strains were harvested from 3-d cultures on Columbia agar supplemented with 7 % horse blood and suspended in 2 mL of Brucella broth. Suspension (0.5 mL) with a concentration of standard 3 according to the McFarland scale was swabbed onto each plate and applied to E-test strips. The strips were saturated with antibiotics at 0.016–256 mg/L. The plates were incubated at 37 °C under microaerophilic conditions (5 % O₂, 10 % CO₂, 85 % N₂) for 3 d. The strains were considered resistant to Met, Cla and Amo when the MIC were >8, >1, and >0.5 mg/L, respectively.

Treatment. During 5 years of observation, the group of children underwent eradication therapy with different combinations of drugs, which varied according to the results of the *H. pylori* susceptibility tests. The patients were treated with proton pump inhibitors (e.g., Ome) in combination with 2 antibiotics out of...
the following: Amo, Cla or Met, for 7 or 10 d. Treatment efficacy was evaluated 6 weeks after completion of therapy. Eradication of *H. pylori* was assessed on the basis of the culture of gastric biopsy specimens obtained during control endoscopic examination, which was performed 6 weeks after completion of therapy.

**Statistical method.** Statistical evaluation was carried out by the $\chi^2$-test, the results being considered significantly different at $p < 0.05$.

**RESULTS**

*H. pylori* strains were isolated from 539 gastric biopsies. Of these, 409 were from untreated and 130 from treated pediatric patients. Strains isolated from the patients after treatment showed a significantly higher Met- and Cla-resistance than those isolated before treatment (Met 48.4 % vs. 35.2 %, $\chi^2 = 7.33, p < 0.01$; Cla 17.6 % vs. 8.6 %, $\chi^2 = 8.57, p < 0.01$) (Table I). Of 539 strains examined, 38.4 % were resistant to Met and 10.7 % to Cla. All strains were sensitive to Amo.

There was no statistically significant difference in the Met resistance among the strains isolated in 1999–2001 ($p > 0.1$) (Table II). Among the strains isolated before treatment, the frequency of Cla-resistant strains increased from 5.3 % in 1999 and 4 % in 2000 to 15 % in 2001 ($\chi^2 = 6.09, p < 0.05$). The frequency of Cla-resistant strains which were isolated after treatment was also the highest in 2001. The increase, however, was not statistically significant ($p > 0.1$). The proportion of strains resistant to both Cla and Met, which was 3.8 % in 1997–2000, increased to >11.1 % in 2001 ($p < 0.01$).

The effect of the administered antibiotics on the proportion of Cla- and Met-resistant strains was examined using isolates from 130 pediatric patients after treatment (Table III). The strains obtained after therapy was classified into 3 groups according to the treatment: Ome + Amo + Met, Ome + Amo + Cla and Ome + Cla + Met. Cla-resistant strains were isolated more frequently from patients treated with Ome + Amo + Cla than from those treated with Ome + Amo + Met (9.7 % vs. 22.6 %, $\chi^2 = 9.4$, $p > 0.01$; statistically significant difference). In the Ome + Amo + Met and Ome + Amo + Cla groups, the frequency of Met-resistant strains was 56 and 45 %, respectively (but the difference between the two groups was not significant; $p > 0.1$). The rate of Met resistance was higher for girls (44 %) than for boys (33 %) ($p < 0.05$), but there was no significant difference in the rate of Cla resistance (Table IV).

**DISCUSSION**