Antibiotic-Resistance Patterns of *Helicobacter pylori* in Croatia: Cohort Study

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**Aim.** To provide information on regional sensitivity of *H. pylori* to antibiotic treatment by investigating the rate of *H. pylori* eradication in Croatia.

**Methods.** The study included 217 outpatients (107 women and 110 men), with gastrointestinal symptoms and *H. pylori* positive finding. They received the first-line and second-line treatments. The first-line treatment included triple therapies with either omeprazole (omeprazole, amoxicillin, and metronidazole – OAM), or pantoprazole (pantoprazole, amoxicillin, and metronidazole – PAM), or a combination of ranitidine bismuth citrate, amoxicillin, and azithromycin (RBAAz). If this therapy failed, clarithromycin was used in the second-line treatment.

**Results.** *H. pylori* was eradicated in 93% of the patients, whereas in 7% it was resistant to all administered therapies. The efficacy of OAM or PAM first-line treatments in *H. pylori* eradication, including resistant patients, was 70%, and of RBAAz treatment 95%. The RBAAz treatment had the highest eradication rate. In the second-line treatment, clarithromycin eradicated 45% of the remaining *H. pylori* strains that had not reacted to metronidazole and azithromycin administered either alone or in combination with ranitidine bismuth citrate.

**Conclusion.** Optimal therapy for the eradication of *H. pylori* infection is the RBAAz treatment, whereas metronidazole cannot be recommended because of the high rate of resistance of *H. pylori* to that antibiotic.

**Key words:** amoxicillin; azithromycin; clarithromycin; drug resistance, microbial; drug therapy, combination; gastritis; *Helicobacter pylori*; metronidazole; omeprazole; peptic ulcer; ranitidine bismuth citrate

As a transmissible, widespread disease that causes progressive damage to the stomach, *H. pylori* infection of the gastric mucosal surface represents a great gastroenterological problem. In spite of the wealth of published data, an optimal therapy for a specific geographical region or social conditions (1-4) is often difficult to find due to the rapid changes in the epidemiological picture of this disease. Moreover, most of the randomized controlled studies (1-8) have not investigated the second-line treatment (2-10) of *H. pylori* infection.

Several studies on different antimicrobial combinations for the treatment of *H. pylori* infection have been carried out in Croatia (9,10) but none provided a general epidemiological picture, assessed the extent of the problem, or clearly recommended the therapy for *H. pylori* eradication.

In this study, we followed 217 patients from Zagreb and neighboring areas who had gastric symptoms and, after being diagnosed with the infection in gastroenterological endoscopic outpatient clinics, underwent the treatment for *H. pylori* infection during 1999. The aim of the study was to determine the eradication rate of *H. pylori* infection and the post-treatment *H. pylori* resistance in a group of treated patients, and to define the optimal therapy with respect to the current eradication rate in Croatia.

**Patients and Methods**

**Patients**

All patients admitted to our gastroenterological outpatient clinic for a gastroscopic examination between February 1999 and February 2000 were included in this cohort study. All had gastroenterological symptoms and were *H. pylori* positive. Out of 297 *H. pylori* positive patients who entered the study, 80 discontinued the treatment, stating the fear of gastroscopy as the main reason for discontinuation. However, as *H. pylori* infection was verified in each patient on the basis of gastric biopsy samples, gastroscopy could not have been avoided.

Finally, 217 patients with positive *H. pylori* diagnosis were included in the analysis, 107 women (median age 56 years, range 23-80) and 110 men (median age 51 years, range 18-78). In these patients, *H. pylori* was either eradicated or labeled as resistant at the completion of the therapy.
Patients were divided into four groups according to their endoscopic diagnoses: (a) duodenal ulcer disease (active or in remission), (b) gastric ulcer, (c) active gastritis or gastric erosion, and (d) normal finding. Normal endoscopic finding, as a macroscopic diagnosis, does not exclude the possibility of, in some degree, active chronic gastritis, which can be histopathologically diagnosed, and the presence of the H. pylori strain. For this reason, biopsy specimens of gastric mucosa were taken from the patients with both gastroenterological symptoms and with normal endoscopic findings. The diagnosis of H. pylori infection was established by two standard tests, the histopathological analysis and the rapid urease test. All gastric tissue samples related to gastric pathology were histopathologically analyzed, whereas the rapid urease test, as a standard gastroenterological procedure, was used in follow-up examinations.

Types of Therapy

The medicaments for the combination therapy were chosen from the group of medicaments recommended by the Croatian Gastroenterological Society and included in the list of medicaments covered by the Croatian Institute for Health Insurance (11).

During the spring and summer of 1999, the combination of either omeprazole (20 mg), amoxicillin (1,000 mg), and metronidazole (400 mg) (OAM), or pantoprazole (40 mg), amoxicillin (1,000 mg), and metronidazole (400 mg) (PAM) was administered as the first-line therapy twice a day for a week.

During the autumn and winter of 1999, the combination of ranitidine bismuth citrate (400 mg), amoxicillin (1,000 mg), and azithromycin (1,000 mg) (RBAAz) was used as the first-line treatment. Ranitidine bismuth citrate and amoxicillin were administered twice a day for a week, whereas azithromycin was administered for three days.

Therapy sequences were chosen so that different groups of medicaments could be applied over different seasons (spring or autumn), because the season can biologically influence the course and frequency of dyspeptic diseases.

When H. pylori could not be eradicated with the first-line treatment, the second-line treatment was administered as follows:

- As shown in the Figure 1, the RBAAz first-line therapy, 95% successful in eradication of H. pylori infections, was significantly more efficient than the OAM or PAM first-line therapy, which eradicated only 70% of H. pylori infections (chi-square=10.57, p=0.001).

The second-line treatment was used in 48 patients in whom H. pylori could not be eradicated with the OAM or PAM first-line treatments. As a part of the first and second phase of treatment, azithromycin or RBAAz eradicated H. pylori in 46% (22/48) of those patients (Fig. 1).

Three patients who did not have H. pylori eradicated with the RBAAz first-line treatment entered the second-line treatment that included clarithromycin. The bacteria was eradicated in two of them, but remained resistant in the third patient (Fig. 1). There was only one case of resistance in the group of 56 patients treated with RBAAz therapy sequence, whereas in the group of 161 patients treated with OAM or PAM, 15 were resistant.

The efficacies of OAM and PAM treatments were compared, including the patients in whom H. pylori was not significantly different (p=0.81).
eradiated (Fig. 2). There was no significant difference in \textit{H. pylori} eradication efficacy between the OAM and the PAM first-line therapies (Chi-square=0.06, p=0.81).

\textit{H. pylori} infection was eradicated in 93% of 217 patients who completed the first-line and second-line treatments (Table 1). The remaining 16 patients (7%) were resistant to all the therapies administered.

\section*{Discussion}

In this study, we found high \textit{H. pylori} resistance to metronidazole, which renders it unsuitable for treating \textit{H. pylori} infection. On the other hand, azithromycin, a macrolide antibiotic, showed a high eradication rate in combination with ranitidine bismuth citrate and amoxicillin. Therefore, it can be recommended for \textit{H. pylori} eradication.

Important issues in \textit{H. pylori} eradication are the influence of antisecretory drugs and negative aspect of \textit{H. pylori} resistance to antimicrobial agents. The prevalence of antibiotic-resistant \textit{H. pylori} strains is on the increase and presents one of the main causes of treatment failure (5). The best way to prevent the emergence of bacterial resistance is to reach the highest possible eradication rate. The eradication rates achieved in routine clinical practice are similar to those found in randomized controlled clinical trials (12).

This study is not a randomized controlled trial, but rather an observational study, whose scope and design were dictated by the availability of the antibiotics. The results show that there was no significant difference in \textit{H. pylori} eradication rates between omeprazole and pantoprazole triple therapies (OAM or PAM) in the first-line treatment. Another Croatian study (9) reported similar results for the OAM treatment, but for the PAM treatment their results were significantly higher than those obtained in our study.

Some studies reported 21-45\% primary metronidazole resistance, which clearly had a negative impact on the eradication rate in the OAM and PAM triple therapies (2,5,12-16). Hence, treatments including metronidazole should be avoided in the population with high rates of metronidazole resistance.

On the other hand, our study showed that the RBAAz therapy in the first-line treatment achieved the highest eradication rate of 95%.

Our results on the efficacy of treatment with azithromycin are similar to the results of another Croatian group (9), but better than results of a study carried out by Tarisoli et al (17), who reported on the 81\% eradication rate after a four-day low-dose therapy with azithromycin in combination with different medications (lansoprazole, azithromycin, and tinidazole).

Except being more effective in the first-line treatment, azithromycin provides additional eradication in the OAM or PAM sequence after the unsuccessful first-line treatment by a combination including metronidazole. In our study, the efficacy of RBAAz proved considerably higher in the first-line (95\%) than in the second-line treatment, when azithromycin was administered after the OAM or PAM first-line treatments (46\%). This is obviously a consequence of the resistance-creating effect of metronidazole that was used in the first-line treatment. Lund et al (5) recognized the same effect. They showed that the application of metronidazole before clarithromycin reduces the efficacy of clarithromycin, i.e., it induces some degree of antibiotic resistance in \textit{H. pylori}. Thus, beside provoking primary resistance in the first-line treatment, metronidazole clearly exhibits a cross-reaction resistance with azithromycin in the second-line treatment.

Clarithromycin, administered as the antimicrobial agent in the last phase of the second-line treatment, eradicated 45\% of \textit{H. pylori} that had reacted neither to metronidazole nor to azithromycin in combination with ranitidine bismuth citrate or amoxicillin. Most probably, the effect of clarithromycin would have been better if it had been used in the first-line treatment, because its primary resistance does not exceed 3\%, as shown in some recent studies (5,16). For this reason, other multi-center studies (2-7) also used it in the first-line treatment. However, since the Croatian Institute for Health Insurance did not include clarithromycin in the official list of medications before this study was completed, it was not often administered in treatment of \textit{H. pylori} and other infections.

Although clarithromycin had been widely recommended for the first-line treatment and its efficacy in \textit{H. pylori} eradication proven (2-7), the Croatian Institute for Health Insurance did not cover it in time when this study was carried out. Clarithromycin was included in the list not long after this study had finished.

A particular problem that showed up in this study was the 7\% post-treatment resistance of \textit{H. pylori}. Of 16 post-treatment \textit{H. pylori} resistant patients, more than a half had duodenal ulcer disease. As they had been previously treated with antisecretory drugs and antimicrobial agents, their strains of \textit{H. pylori} could have developed resistance. However, it is interesting that two \textit{H. pylori}-resistant patients had normal endoscopic findings but active chronic gastritis histopathologically, and had not been previously exposed to these medications.

Antimicrobial resistance has a significant effect on the outcome of the therapy. It should be emphasized that antimicrobial-resistant strains of \textit{H. pylori} might soon flourish in the general population because of the both increasing number of patients who require therapy and sub-optimal regimes that are being prescribed. Inadequate choice of the combination of medications can make the therapy more expensive. At the same time, it adversely affects the treatment of \textit{H. pylori} infection by increasing the risk of resistant strain development, turning physicians’ efforts into the labor of Sisyphus.
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References


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