

Antimicrobial Resistance of *Helicobacter pylori* Clinical Strains in the Last 10 Years

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Abstract

In the present work primary antimicrobial resistance was analyzed in clinical *H. pylori* strain isolates from adult patients from Polish Wielkopolska region within the last 10 years. Drug sensitivity was evaluated in a total of 142 *H. pylori* isolates, with 66 strains originating from years 1997/1998 forming group 1 and 76 strains isolated in 2007/2008 forming group 2. Sensitivity to amoxicillin, tetracycline, metronidazole and clarithromycin was determined by E-test. All strains were susceptible to amoxicillin and tetracycline. On the other hand, a high proportion of strains resistant to metronidazole was determined (36.4% in group 1 and 44.7% in group 2). In parallel, a growing tendency was discovered for resistance to clarithromycin (9.1% strains resistant in group 1 and 18.4% isolates resistant in group 2). The studies confirm the need for monitoring the drug resistance of *Helicobacter pylori* strains.

Key words: *Helicobacter pylori*, clinical isolates, drug resistance

Introduction

Helicobacter pylori is a highly prevalent pathogenic bacterium, which colonizes the gastric mucosa, inducing chronic inflammation, which in consequence may cause peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma (MALT) and, most significantly, gastric adenocarcinoma (Konturek *et al.*, 2003; Perez-Perez *et al.*, 2004). In anti-*H. pylori* therapy it is recommended to apply combined regimens, consisting of an acid-suppressive drug or a bismuth component with two or more antimicrobial agents, such as clarithromycin, metronidazole, amoxicillin or tetracycline (Mégraud and Lamouliatte, 2003; McLoughlin *et al.*, 2004). Nevertheless, the eradication therapy is not always successful for this pathogen (Malfertheiner *et al.*, 2002). One of the most important reasons for treatment failure is the development of *H. pylori* resistant to antimicrobials. The prevalence of *H. pylori* resistance varies geographically and changes dynamically (Mégraud, 2004) and, therefore, requires constant monitoring.

In view of the above, our studies aimed at an analysis of primary antimicrobial resistance in clinical *H. pylori* strains originating from adult patients from Wielkopolska region of Poland within the last 10 years.

Experimental

Materials and Methods

Bacterial isolates. Evaluation of drug sensitivity was performed on the total of 142 strains of *H. pylori*, isolated before treatment from gastric mucosa of adult patients, diagnosed in the Department of Medical Microbiology, University of Medical Sciences in Poznań, Poland. Out of them, 66 strains isolated in years 1997/1998 formed group 1 and 76 strains isolated in 2007/2008 formed group 2. Biopsies isolated from the prepyloric portion were immediately placed in a transport medium (Portagerm pylori; bioMerieux, Marcy-l'Etoile, France). The obtained biopsies were plated on Columbia agar, supplemented with 7% sheep blood and a set of antibiotics (*H. pylori* selective supplement Dent SR 147E, Oxoid, Basingstoke, UK). The incubation was performed in microaerophilic conditions (Generbag or Generbox microaer, bioMerieux) for 4–7 days at the temperature of 37°C. The grown out strains were identified basing on colony morphology, staining according to Gram, ability to produce urease and catalase.

Before the drug resistance test, a few of the grown colonies were plated on Columbia agar supplemented

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with 7% sheep blood and incubated for subsequent 3 days in microaerophilic conditions at 37°C.

All the research protocols were reviewed and approved by the Ethics Committee of the University of Medical Sciences in Poznan, Poland.

Antimicrobial susceptibility testing. For the drug susceptibility test a suspension of grown bacteria was used, in PBS, manifesting density 2 in McFarland's scale. Sensitivity to amoxicillin, clarithromycin, metronidazole and tetracycline was determined by E-test (AB Biodisc, Solna, Sweden). A strip of the test was placed on Columbia agar supplemented with 7% of sheep blood with pre-plated, examined strain of *H. pylori*. The incubation was performed in microaerophilic conditions, at the temperature of 37°C for 3 days. The following CLSI interpretative criteria were used as breakpoints for *Helicobacter pylori* resistance: clarithromycin (CL) 1 mg/L, metronidazole (MTZ) 8 mg/L, amoxicillin (AMX) 0.5 mg/L and tetracycline (TC)

4 mg/L (Mégraud and Lehours, 2007). The obtained results were presented in the form of MIC₅₀ values (minimum concentration of the antibiotic required to induce growth inhibition in 50% strains) and MIC₉₀ values (minimum concentration of the antibiotic required to induce growth inhibition of 90% strains).

Statistical methods. Statistical analysis was performed using Fisher's exact test. A *p*-value higher than 0.05 was considered non-significant.

Results

Results obtained in both groups of studied strains are presented in Figures 1–4. All the examined strains proved to be sensitive to amoxicillin: in both groups MIC₅₀ and MIC₉₀ values amounted to 0.016 mg/L and 0.023 mg/L, respectively (Fig. 1). All the strains were also sensitive to tetracycline although a change in

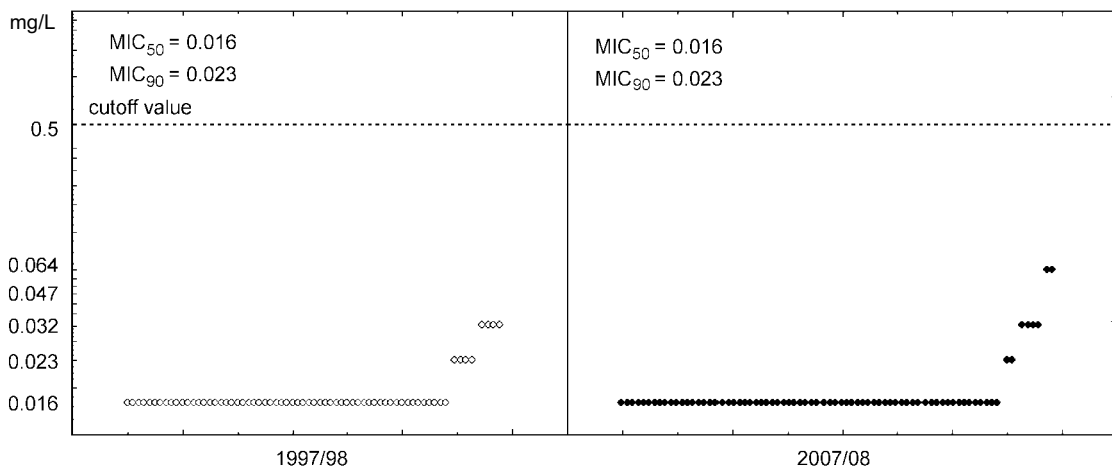


Fig. 1. MIC (mg/L) values of amoxicillin for *H. pylori* strains isolated in the years 1997/1998 and 2007/08.

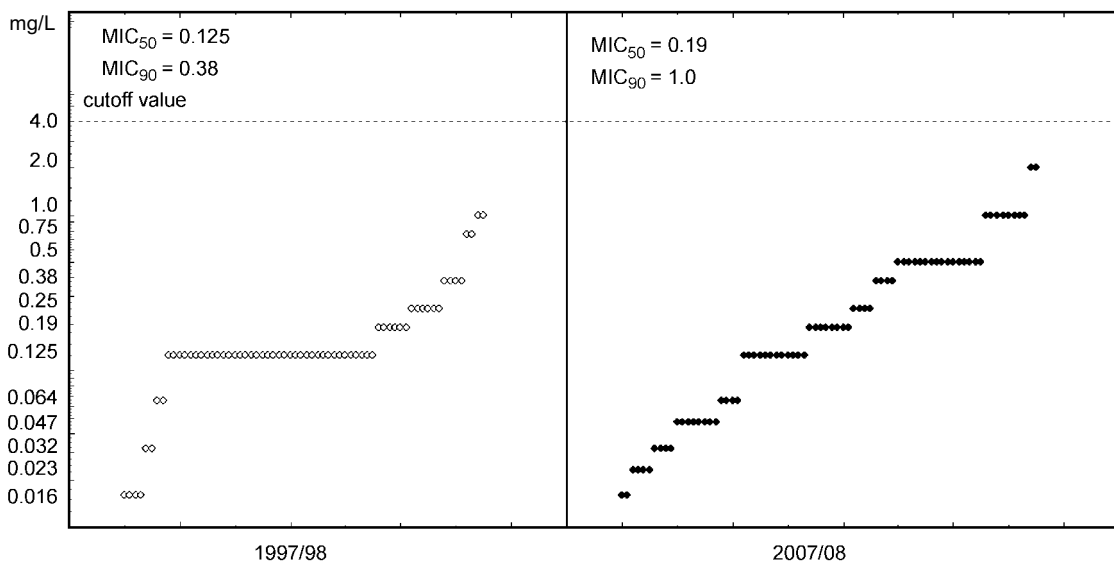


Fig. 2. MIC (mg/L) values of tetracycline for *H. pylori* strains isolated in the years 1997/1998 and 2007/08.

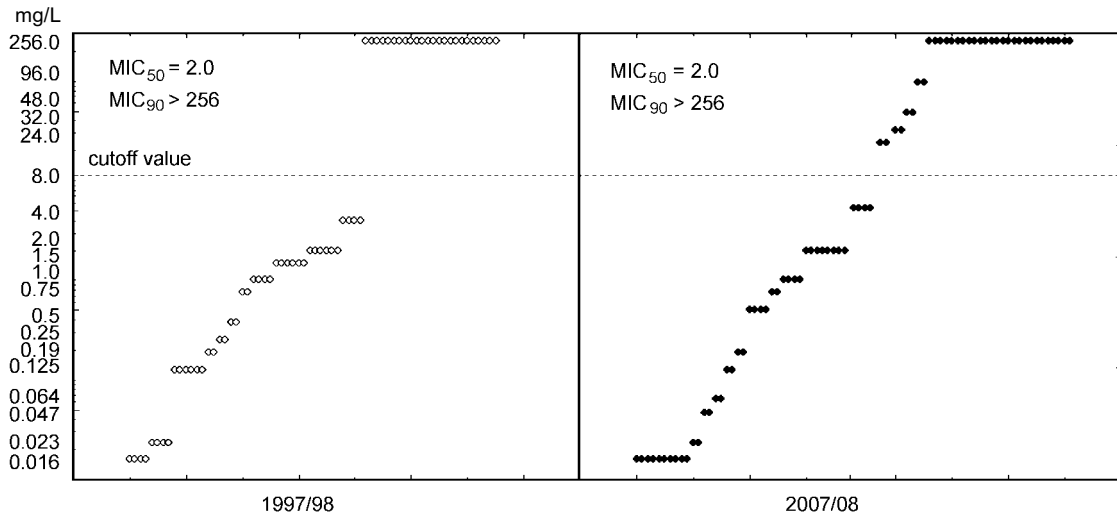


Fig. 3. MIC (mg/L) values of metronidazole for *H. pylori* strains isolated in the years 1997/1998 and 2007/08.

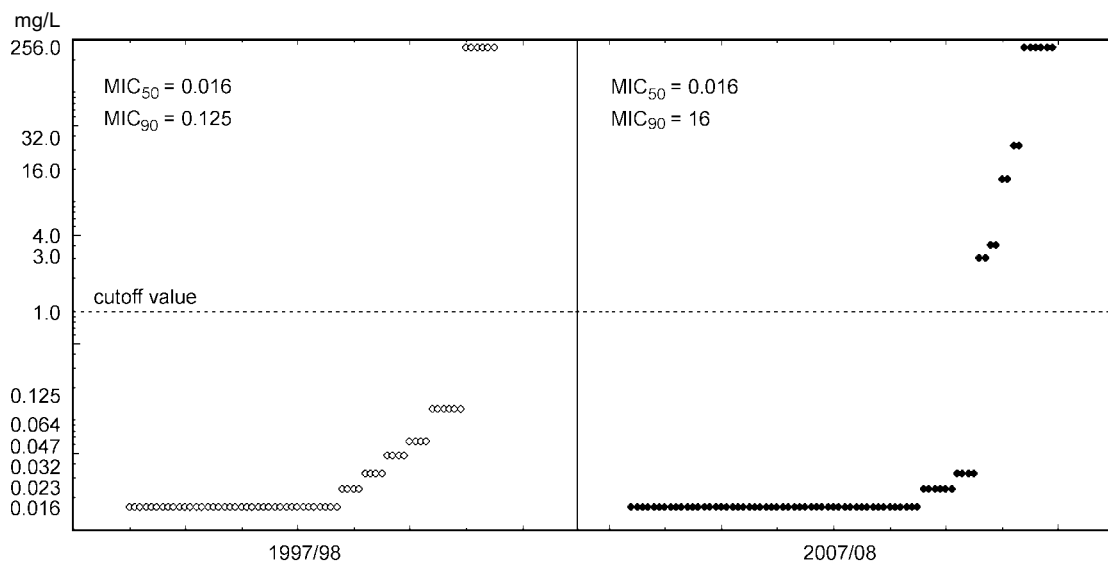


Fig. 4. MIC (mg/L) values of clarithromycin for *H. pylori* strains isolated in the years 1997/1998 and 2007/08.

MIC values between the examined groups was noted. For group 1 strains, values of MIC_{50} and MIC_{90} were 0.125 mg/L and 0.38 mg/L, respectively, while in group 2 they were higher and amounted to 0.19 mg/L and 1.0 mg/L, respectively (Fig. 2).

In the two groups of studied strains a high proportion manifested resistance to metronidazole. In the two groups, the number of resistant strains showed no significant differences amounting to, respectively, 24 (36.4%) strains in group 1 and 34 (44.7%) strains in group 2 ($p=0.5347$). In both groups values of MIC_{50} and MIC_{90} for metronidazole were 2.0 mg/L and >256 mg/L, respectively (Fig. 3).

In studies of resistance to clarithromycin 6 (9.1%) strains were found to be resistant to the drug in group 1 and 14 (18.4%) strains were resistant in group 2 (the difference was insignificant; $p=0.2298$). In all strains

of group 1 manifesting resistance to clarithromycin this was accompanied by resistance to metronidazole. In group 2, on the other hand, in 8 (10.5%) strains resistance to clarithromycin was accompanied by resistance to metronidazole. For clarithromycin, MIC_{50} value amounted to 0.016 mg/L in both groups of studied strains, whereas MIC_{90} were 0.125 mg/L and 16 mg/L in group 1 and 2, respectively (Fig. 4).

Discussion

In the studies drug resistance was evaluated for *Helicobacter pylori* strains isolated at present as compared to strains isolated 10 years ago. The results point to the high prevalence of strains resistant to metronidazole in the Polish population. The problem of resistance

to metronidazole was noted in this country already years ago (Borysiewicz *et al.*, 1993; Andrzejewska and Klincewicz, 1995; Łękowska-Kochaniak and Popowska, 1999) and the present results confirm that such a tendency persists. Among the strains tested by us as many as 44.7% of them proved to be resistant to the chemotherapeutic agent. The results are consistent with earlier multi-centre studies performed in Poland (Rozynek *et al.*, 2002; Dzierżanowska-Fangrat *et al.*, 2005). The problem of resistance to metronidazole has also been observed in other countries. In European countries, in Asian countries and in USA 20% to 40% of strains are noted to be metronidazole-resistant and such strains have been found to be the least frequent in the North of Europe (Loffeld and Fijen, 2003; Lui *et al.*, 2003; Cameron *et al.*, 2004; Mégraud, 2004; Janssen *et al.*, 2006; Chisholm *et al.*, 2007). A slightly lower proportion of such strains has been detected in Japan (9 to 12%) and in Canada (18–22%) (Mégraud, 2004). The increasing tendency for drug resistance, noted both in our studies and in investigations of other authors (Cameron *et al.*, 2004; Chisholm *et al.*, 2007) is disquieting. Even higher percentages of metronidazole-resistant strains of *H. pylori*, between 50% and 80% strains, were noted in developing countries (Loffeld and Fijen, 2003; Mégraud, 2004), which was linked to broad application of the drug for eradication of parasitic infections. The frequent application of metronidazole in the treatment of gynaecological, dental diseases and ineffective treatment of *H. pylori* infections markedly increases the risk of metronidazole resistance (Gerrits *et al.*, 2006). Also in the case of clarithromycin the disquieting tendency for drug resistance has been noted. In our studies we have documented the number of such strains resistant to the antibiotic to double as compared to the years of 1997/98. Such an unfavourable evolution of drug resistance has been noted in many other countries, the European ones, in the United States and in Japan (Loffeld and Fijen, 2003; Cameron *et al.*, 2004; Masuda *et al.*, 2004; Francesco *et al.*, 2006; Janssen *et al.*, 2006; Chisholm *et al.*, 2007). Even more extensively clarithromycin resistance is observed among strains isolated from children (Rozynek *et al.*, 2002; Mégraud, 2004; Dzierżanowska-Fangrat *et al.*, 2005), which is linked to broad application of macrolide antibiotics in paediatrics.

Macrolide antibiotics, particularly those of the new generation, are applied with increasing frequency also in adults to eradicate infections induced by chlamydiae and mycoplasma. Perhaps the progress in treatment of the infections is responsible for development of clarithromycin-resistant strains.

It is worth particular attention that parallel manifestation to clarithromycin and to metronidazole de-

velops within the same strains (in our studies this has been observed in 9.9% all tested strains), which causes that the combined therapy of the two antibiotics as a therapeutic option in eradication of *H. pylori* becomes problematic.

Sensitivity to the remaining antibiotics, amoxicillin and tetracycline persists but the observed by us evolution of MIC values for tetracycline within 10 years of observation may promote the development of *Helicobacter pylori* strains resistant to the antibiotic.

It is therefore recommended to continue monitoring of drug resistance in *Helicobacter pylori* strains to appropriately verify therapeutic options.

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