

The Antimicrobial Susceptibility of *Helicobacter pylori* Strains Isolated from Children and Adults with Primary Infection in the Lower Silesia Region, Poland

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Abstract

The resistance of microorganisms to antibiotics has become a serious issue in recent years in the therapy of bacterial infections. This problem also concerns the treatment of infections caused by *Helicobacter pylori* strains. The aim of this study was to evaluate the frequency of primary resistance of *H. pylori* strains isolated from children and adults. The subject of the research was 105 strains of *H. pylori* isolated from children and 60 strains from adults in the Lower Silesia Region in the years 2008–2011. Antimicrobial susceptibility to the following antibiotics was assessed: amoxicillin (AC), clarithromycin (CH), metronidazole (MZ), tetracycline (TC), levofloxacin (LEV) and rifabutin (RB). Among the strains isolated from children, 33.3% were resistant to CH, 44.8% to MZ whereas 1.9% of strains were resistant simultaneously to CH, MZ and LEV. Among 60 strains isolated from adults, 23.3% were resistant to CH, 66.7% to MZ, and 6.7% to LEV. Moreover, 16 multidrug resistant strains were isolated from adults, including 12 resistant to CH and MZ, 3 to MZ and LEV, and 1 to CH, MZ and LEV. All examined strains were susceptible to AC, TC and RB. The high incidence of resistance to CH and MZ suggests that standard triple therapies may not be useful as first-line treatment in Poland without earlier susceptibility testing.

Key words: *Helicobacter pylori*, adults, children, resistance

Introduction

Colonization of the human gastric mucosa with *Helicobacter pylori* induces chronic gastritis and peptic ulcer disease. In addition, *H. pylori* plays a role in pathogenesis of gastric cancer and cancer of the mucosa-associated lymphoid tissue (Mishra, 2012). Treatment of *H. pylori* infection usually includes a combination of two antimicrobial agents (among amoxicillin, metronidazole and clarithromycin) and a proton pump inhibitor or bismuth salts. The ideal antimicrobial therapy should have an eradication rate of at least 90% and a low incidence of significant side effects and the drugs should be available worldwide. However, more recent publications have suggested that this level has fallen alarmingly to around 70% in many areas and even as low as 60% in some other regions (Kadayifci *et al.*, 2006). Attempts are being made to use traditional therapeutic patterns in combination with various probiotics, *e.g.* *Lactobacillus acidophilus* (Da Silva Mendeiros *et al.*, 2011). The success of the therapy depends on

several factors, but one of the most important seems to be the increasing resistance of *H. pylori* strains to antibiotics (Glupczynski *et al.*, 2001). Drug susceptibility of *H. pylori* strains is changeable both in different regions of the world and in different regions of the same country, so it is essential to have knowledge of the local profile of drug susceptibility of microorganisms in order to choose the most effective therapy. The aim of this study was to assess the primary resistance of *H. pylori* strains isolated from children and adults in the Lower Silesia Region in Poland.

Experimental

Material and Methods

The study was performed on 165 strains isolated from pediatric and adult patients of the Lower Silesia Region in the years 2008–2012. Our study involved 105 children aged 4–18 and 60 adults aged 19–89 who

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underwent endoscopic examination of the upper gastrointestinal tract due to complaints from the upper gastrointestinal tract, such as abdominal pain, nausea, or vomiting suggesting the presence of pathology. Patients had not been previously diagnosed and treated for *H. pylori* infection. Patients who had previously had *H. pylori* infection or received antibiotics within the last 2 months were excluded. Other exclusion criteria were parasitic diseases, allergies and autoimmune diseases. Informed written consent was obtained from each patient. The study was approved by the Bioethics Committee of Wrocław Medical University, Approval No. 226/2011. Biopsies from the antrum and, in the case of present changes, from the corpus were taken from each patient during endoscopy of the upper gastrointestinal tract for histopathology and microbiology. Biopsies collected for microbiological examination were placed immediately after collection in sterile saline (0.15 M NaCl) and processed within two to three hours in a microbiological laboratory. The isolation and identification of strains were performed as described previously. [5] After the primary isolation and identification, the strains were kept frozen at -70°C in Brucella broth containing 15% glycerol. Then the drug to six antibiotics- amoxicillin (AM), clarithromycin (CH), metronidazole (MZ), tetracycline (TC), levofloxacin (LEV) and rifabutin (RB) sensitivity – was determined by gradient diffusion (E-test, BioMerieux) with the method described by Glupczynski *et al.* (2001). Criteria for interpretation of results were as follows: MIC ($\mu\text{g}/\text{mL}$) for resistant strains: amoxicillin >0.5 , clarithromycin >1 , metronidazole >8 , tetracycline >1 , levofloxacin >0.5 and rifabutin >1 (Glupczynski *et al.*, 2001; Megraud and Lehours, 2007).

Statistical analysis was performed by chi-square test with or without Yates' correction and chi-square test among age groups. A p value <0.05 was considered significant for all tests.

Results

The total resistance of strains isolated from adults and children is shown in Table I. Among the strains isolated from children, susceptibility was indicated in 40 (38.1%) strains and 65 (61.9%) were resistant. Among the resistant strains, 35 (33.3%) were resistant to CH, including 18 (17.1%) resistant only to CH, 15 (14.3%) to CH and MZ and 2 (1.9%) to CH, MZ and LEV. Resistance to MZ was detected among 47 (44.8%) strains, 30 (28.6%) were resistant only to MZ and the others were multidrug resistant. Among 60 strains isolated from adults, 42 (70%) were resistant to at least one antibiotic, including the total resistance to CH amounting to 23.3% ($n=14$), to MZ 66.7% ($n=40$), and to LEV

Table I
A comparison of the frequency of primary resistance of *H. pylori* isolated from children and adults.

Resistance	Children (105)		Adults (60)		Total (165)	
	No.	%	No.	%	No.	%
CH (total)	35	33.3	15	25.0	50	30.3
CH (single)	18	17.1	0	0.0	18	10.9
CH+MZ	15	14.3	13	21.7	28	17.0
CH+MZ+LEV	2	1.9	2	3.3	4	2.4
MZ (total)	47	44.8	42	70.0	89	53.9
MZ (single)	30	28.6	24	40.0	54	32.7
MZ+CH	15	14.3	13	21.7	28	17.0
MZ+CH+LEV	2	1.9	2	3.3	4	2.4
MZ+LEV	0	0.0	3	5.0	3	1.8
LEV (total)	2	1.9	7	11.7	9	5.5
LEV (single)	0	0.0	2	3.3	2	1.2
MZ+CH+LEV	2	1.9	2	3.3	4	2.4
MZ+LEV	0	0.0	3	5.0	3	1.8

No – number, CH – clarithromycin, MZ – metronidazole, LEV – levofloxacin

6.7% ($n=4$). *H. pylori* strains resistant to levofloxacin were resistant in 75% to clarithromycin and/or metronidazole. Moreover, 16 multidrug resistant strains were isolated from adults, including 12 resistant to CH and MZ, 3 to MZ and LEV, and 1 to CH, MZ and LEV. No strains were resistant to AM, TC and RB (Table II).

In order to analyze the drug resistance of *H. pylori* strains depending on age, patients were divided into 6 age groups (2–7, 8–12, 13–18, 19–44, 45–64, >65 years) (Fig. 1). The resistance of strains to clarithromycin was the highest among children aged 13–18; the percentage of strains resistant to this antibiotic decreases with age. However, in the case of metronidazole, a gradual increase of resistant strains was found, the highest percentage being noted in the group aged 45–64 (78%). Strains resistant to levofloxacin were found among the oldest children and in the group of adults aged 45–64 (14.8%). The percentage of strains resistant to both CH and MZ remained steady among children in all age groups (14–16%) and adults (18.5–25%). Strains resistant to CH, MZ and LEV were observed in the group aged 14–64 at a similar level (3.7–4.8%).

Discussion

In the last decade one of the most important problems of contemporary medicine has become the resistance of micro-organisms to antibiotics. This problem does not exclude *H. pylori* bacilli. It is acknowledged that the appropriate course of antibiotic therapy contributes to the eradication of this micro-organism in

Table II
In vitro activity of six antimicrobial agents against *H. pylori*

Anti-biotic	Resistant strains				Range
	No.	% (95% confidence interval)	MIC ₅₀	MIC ₉₀	
CH	50	30.3 (26.9–33.7)	0.016	1.5	0.016–256
MZ	89	53.9 (44.41–63.47)	16	126	0.016–256
LEV	9	5.5 (3.69–7.22)	0.064	0.125	0.002–32
AM	0	0 (0.0–0.0)	0.016	0.016	0.016–256
RB	0	0 (0.0–0.0)	0.02	0.02	0.002–32
TC	0	0 (0.00–0.01)	0.023	0.047	0.016–256

No - number, CH - clarithromycin, MZ - metronidazole, LEV - levofloxacin, AM - amoxicillin, TC - tetracycline, RB - rifabutin, MIC₅₀ - Minimum Inhibitory Concentration required to inhibit the growth of 50% of organisms, MIC₉₀ - Minimum Inhibitory Concentration required to inhibit the growth of 90% of organisms

about 80% of patients (Selgrad and Malfertheiner, 2011). According to Graham and Shiotani an effective antibiotic therapy should contribute to the eradication of this micro-organism in 90–94% of patients and an excellent therapy ≥95% (Graham and Shiotani, 2008). The phenomenon of drug resistance of *H. pylori* strains is significant in the case of eradication therapy. In the classical therapy (IPP + AC+CH or IPP + MZ + CH) the most significant is *H. pylori* resistance to clarithromycin, which, with maintained susceptibility to MZ, lowers the effectiveness of eradication to 66% and 35% respectively (Megraud, 2004). Multicenter studies conducted in 2008–2009 by Megraud *et al.* concerning the primary resistance of *H. pylori* strains indicated that 31.8% of strains isolated from children and 17.5% of strains

isolated from adults were resistant to clarithromycin. However, the resistance of strains to metronidazole was 25.7% and 34.9% respectively (Megraud *et al.*, 2013). Our studies have indicated high resistance to clarithromycin among children (33%) and adults (25%). The resistance of examined strains to metronidazole in both groups was considerably higher (average 53.9%). Since the mid-1990s, the gradual increase of *H. pylori* primary resistance to applied antibiotics has been observed. In Poland, the percentage of strains resistant to clarithromycin varies depending on the region, from 21% to 28% (Karczewska *et al.*, 2011, Dzierżanowska-Fangrat *et al.*, 2005, Andrzejewska *et al.*, 2009). A diverse level of resistance to clarithromycin was noted in other countries (5.6–36%). The resistance rates in Europe (11.1%),

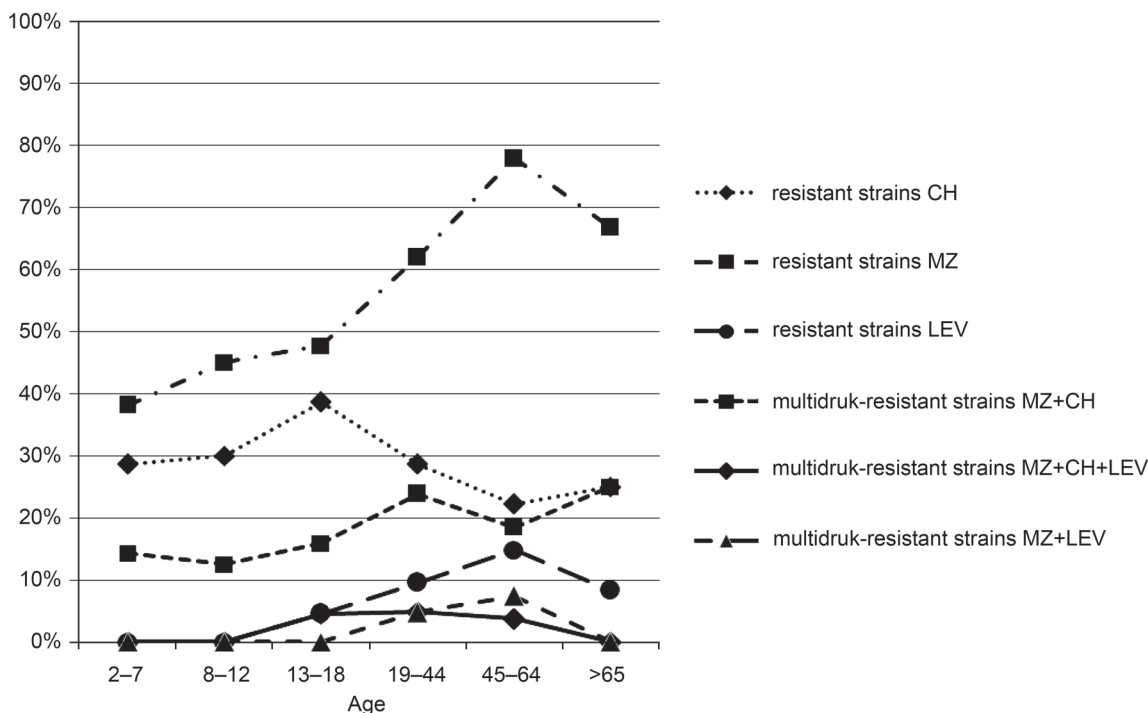


Fig. 1. Resistance of *H. pylori* strains in different age groups.

Asia (18.9%) and America (29.3%) significantly differ (De Francesco *et al.*, 2010). In Europe we can observe large differences in resistance of strains to clarithromycin between northern (10.0%) and southern Europe (20.0%) (Megraud *et al.*, 2013).

Ten years ago, in Poland, the level of strains resistance to metronidazole was in the range 27–52% (average 37%) (Dzierżanowska-Fangrat *et al.*, 2002) and regional variation was observed. Children from the Lower Silesia region have the highest noted resistance, at 52%. A lower percentage of resistant strains could be observed in children from Warsaw (37%), Wielkopolska Region (45%), Kujawsko-Pomorskie Region (20%) and from Łódzki Region (18%) (Bąk-Romaniszyn *et al.*, 2004; Łaszewicz, 2003). The frequency of primary resistance to metronidazole among adults in Europe fluctuated between 28% and 44%. No significant differences were found between southern and northern parts of the continent (respectively – 29.7% and 28.6%). It was noted, however, that the resistance of strains is considerably higher in western and in central Europe (43.8%) (Megraud *et al.*, 2013). In connection with more frequent usage of both clarithromycin and metronidazole in *H. pylori* eradication therapy, the steady increase of strains resistant to two antibiotics at the same time was noted. Towards the end of the 1990s, such resistance was low in Europe and amounted to only 0.8–9.1% (Boyanova, 2009; Elviss *et al.*, 2004). In 2009, in Spain 17.2% of strains were noted to be resistant to both clarithromycin and metronidazole among children (Aqudo *et al.*, 2009). In our study, the percentage of strains primarily resistant to clarithromycin and metronidazole was 14% among children and 21.7% among adults (average 17%). Similar results were obtained in other regions of Poland (13–15.5%) (Dzierżanowska-Fangrat *et al.*, 2005).

The growth tendency also shows the resistance of strains to levofloxacin and currently in Europe, from 7% to 13% of *H. pylori* strains were noted to be resistant. In southern Poland, this resistance level is 12% (Karczewska *et al.*, 2012; Megraud *et al.*, 2013). In our study total primary resistance to LEV was 5.5%, including resistance in children (1.9%) and in adults (11.7%). Moreover, alarmingly, in our region we have observed 1.9% of strains resistant among children and 3.3% among adults to three antibiotics simultaneously. Multidrug-resistant *H. pylori* strains were also observed in other countries, such as Italy, Bulgaria and Taiwan (Boyanova and Mitov, 2010; Yang *et al.*, 2010).

In this work no resistance of *H. pylori* strains to amoxicillin, tetracycline and rifabutin was noted. Other authors also have not found resistance to these antibiotics (Megraud *et al.*, 2013; Karczewska *et al.*, 2011; Gościński *et al.*, 2004). Primary amoxicillin resistance has been low (<2%) in Europe but higher (6–59%) in

Africa, Asia and South America (Boyanova and Mitov, 2010; Yang *et al.*, 2010). Resistance to tetracycline is low (<3%) all over the world, except for Africa, where it amounts to 44% (De Francesco *et al.*, 2010). The observed low resistance to rifabutin so far is very low, 1.3%, and the effectiveness of therapy with rifabutin is good, 73%. Treatment of *H. pylori* infections with rifabutin is promising, especially after failures of eradication with standard antibiotics such as: amoxicillin, metronidazole, clarithromycin, levofloxacin, and tetracycline (Gisbert and Calvet, 2012). However, it should be remembered that the low resistance of *H. pylori* to rifabutin arises from using this antibiotic only to treat *Mycobacterium tuberculosis* (Nishizawa *et al.*, 2011). In conclusion, high incidence of resistance to CH and MZ suggests that standard triple therapies may not be useful as first-line treatment in Poland without earlier susceptibility testing. The use of antibiotics for other indications seems to be the major risk factor for development of primary resistance.

The authors declare no conflict of interest.

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