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Monika M. Biernat[1,A, C, D], Barbara Iwańczak[2, B], Aldona Bińkowska[1, C], Joanna Grabińska[1, B], Grażyna Gościniak[1, E, F]

The Prevalence of *Helicobacter pylori* Infection in Symptomatic Children: A 13-Year Observational Study in the Lower Silesian Region

[1] Department of Microbiology, Wroclaw Medical University, Poland
[2] Department of Pediatric Gastroenterology and Nutrition, Wroclaw Medical University, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. *Helicobacter pylori* (*H. pylori*) infection is very common worldwide, with varying frequency. According to data from epidemiological reports, a third of the population of children in Poland are infected with *H. pylori* before the age of 18. In recent years, a decrease in the incidence of *H. pylori* infection in both children and adults has been observed in many countries.

Objectives. The aim of the study was to assess the prevalence *H. pylori* and non-helicobacter pylori helicobacter (NHPH) infection in symptomatic children from 2000 to 2013, and to analyze the resistance of *H. pylori* strains to antibiotics over the 13-year study period.

Material and Methods. The retrospective analysis was based on the results of cultures for *H. pylori* in 8661 untreated children undergoing their first upper gastroduodenal endoscopy because of clinical symptoms such as chronic abdominal pain/distress, epigastric pain, nausea or vomiting. Drug sensitivity to three antibiotics – amoxicillin (AM), clarithromycin (CH) and metronidazole (MZ) – was determined by the gradient diffusion method (E-test).

Results. Overall, among 8661 cultures analyzed, 16.05% were positive for *H. pylori*. The highest prevalence of infection was found in the year 2000 (23.06%) and the lowest in the year 2010 (8.90%). The prevalence of NHPH infection was low (0.2%). A three-fold increase in the number of strains resistant to both CH and MZ was noted, from 7.9% to about 22.7%. All strains were susceptible to AM.

Conclusions. There has been a decline in the prevalence of *H. pylori* infection in symptomatic children, but this infection is still a common cause of upper gastrointestinal tract symptoms in children treated at the authors' center. Primary antibiotic resistance of *H. pylori* increased over the period analyzed, but primary resistance to metronidazole declined (**Adv Clin Exp Med 2016, 25, 2, 303–308**).

Key words: antibiotic resistance, *H. pylori*, NHPH.

Helicobacter pylori have accompanied humans for thousands of years. Using modern molecular techniques, the genetic material of *H. pylori* was found in the remains of Mexican mummies from the time before Columbus [1]. The isolation of these organisms by R. Warren and B. Marshall in 1983 proved to be a landmark event, changing therapeutic procedures in diseases of the stomach and duodenum [2]. *H. pylori* infection, which occurs mainly in early childhood and with varying frequency, is very common around the world. Factors that influence the incidence of *H. pylori* infection include low socioeconomic status, poverty, inadequate hygiene in childcare and a genetic predisposition. Infection rates in developed countries (20–40%) are much lower than in developing countries (30–70%) [3]. Poland is a country with moderate infection rates: The average incidence of anti-*H-pylori* antibodies in the years 2002–2003 was 58%; it increased with age, from 26% in chil-

dren up to 3 years of age to over 93% in people over 80. According to data from epidemiological reports, a third of the children in Poland are infected before the age of 18 [4].

Among other *Helicobacter* species, non-*Helicobacter pylori* helicobacters (NHPH) play the most important role in infections of the gastrointestinal tract. They can cause similar inflammatory changes as *H. pylori*, and may lead to the development of peptic ulcer disease, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma [5]. In addition, species of the genus *Helicobacter* such as *H. heilmannii sensu stricto*, *H. suis*, *H. felis* and *H. bizozzeronii* have been isolated from people with abdominal pain and vomiting [6]. The main reservoir of *H. pylori* in humans is the digestive tract, but the bacterium has also been found in biopsy samples, dental plaque, saliva and feces [7]. In recent years, a decrease in the incidence of *H. pylori* infection in both children and adults has been observed in many countries [8–10]. The objective of this study was to estimate the prevalence of *H. pylori* infection and NHPH infection in symptomatic children over a 13-year study period, and to analyze the resistance rates of *H. pylori* strains to antibiotics.

Material and Methods

The retrospective analysis was based on the results of *culturea* for *H. pylori* in children aged 1.5––18 years, performed between January 2000 and December 2013. The patient population was homogeneous, of European origin, residing in the Lower Silesian region of Poland. Overall 8661 untreated patients, 4286 girls and 4375 boys undergoing their first upper gastroduodenal endoscopy because of clinical symptoms such as chronic abdominal pain/distress, epigastric pain, nausea or vomiting, were included in the study. Diseases of the liver, pancreas, kidneys, parasitic infestation or evidence of any other viral or bacterial infections were excluded in all the children. Informed written consent was obtained from all the parents of children and from patients older than 16 years of age who underwent invasive diagnostic procedures. Four gastric mucosal biopsy specimens (two from the antrum and two from the corpus) were taken from each child for histopathological and microbiological examination. The samples were evaluated histologically according to the Sydney classification [11]. *H. pylori* infection was defined as a positive culture for *H. pylori* and the presence of inflammation in the gastric mucosa (*H. pylori*-associated gastritis). The bacteriological examination consisted of direct microscopy assessment, culture and susceptibility testing as described elsewhere [12]. Briefly, the strains were identified as *H. pylori* by Gram stain morphology, a positive culture and positive catalase, oxidase and urease tests. NHPH identification was based on the presence of strongly spiral bacteria in a direct sample and lack of growth on selective media. Drug sensitivity to three antibiotics – amoxicillin (AM), clarithromycin (CH) and metronidazole (MZ) – was determined by the gradient diffusion method (E-test, BioMérieux, Marcy-l'Étoile, France) and the method described by Glupczynski et al. [13]. To interpret the results, the criteria for resistant strains were minimum inhibitory concentrations (MIC, μg/mL) of amoxicillin > 0.5, clarithromycin > 1, and metronidazole > 8 [13, 14]. Statistical analysis was performed by χ^2 test with or without Yates' correction. A p-value of < 0.05 was considered significant for all the tests. Statistical analyses were done by using STATISTICA v. 10.0 software (StatSoft).

Results

Overall, among the 8661 cultures analyzed over the 13-year study period, 1390 (16.05%) were positive for *H. pylori*. The prevalence of infection was similar in girls (663/4286) and boys (693/4375) (15.5% vs. 15.8%, $p > 0.05$). The prevalence of *H. pylori* infection in certain years is presented in Table 1. The highest prevalence of *H. pylori* infection was noted in 2000 (23.06%), and the lowest in 2010 (8.9%). The prevalence of *H. pylori* infection in the period from 2009 to 2011 was significantly lower than from 2000 to 2002 (10.02% vs. 21.49%, $p < 0.001$), and also lower than in 2012–2013 (23.42% vs. 13.65%, $p < 0.001$).

The prevalence of *H. pylori* infection in different age groups was also analyzed in two time periods: 2000–2001 and 2010–2011. The patients were aged 1.5–4 years (n = 142), 5–8 years (n = 537), 9–11 years (n = 459), 12–14 years (n = 515) and 15–18 years (n = 513). In the years 2000–2001, *H. pylori* infection was found in 4/20 children aged 1.5–4 years (20%), in 47/234 subjects in the group aged 5–8 years (20%), in 47/242 children aged 9–11 (19%), in 79/311 children aged 12–14 years (25%), and in 85/316 children aged 15–18 years (27%). In 2010–2011, the prevalence of *H. pylori* infection was significantly lower in patients from the group aged 1.5–4 years, (27/303, 9%, $p < 0.01$) and in children aged 5–8 years (24/217, 11%, $p < 0.01$). In children aged 9–11 years, *H. pylori* infection was noted in 5/122 cases (4%), in 34/204 subjects aged 12–14 years (17%), and in 30/197 of the 15–18-year-olds (15%), but the differences

Table 1. Annual rates of *H. pylori* and NHPH infection

Year	No. of children	No. and percent of isolated *H. pylori* strains		No. of NHPH	
		n	%	n	%
2000	529	122[A]	23.06	0	0
2001	668	140[A]	20.96	2	0.3
2002	902	189[A]	20.95	1	0.1
2003	694	124[A]	17.87	0	0
2004	740	159	21.49	2	0.3
2005	701	118	16.83	3	0.4
2006	785	129	16.43	2	0.2
2007	782	114	14.58	2	0.2
2008	814	81	9.95	1	0.1
2009	667	70	10.49	1	0.1
2010	730	65[B]	8.90	1	0.1
2011	400	45[B]	11.25	0	0
2012	141	19[B]	13.47	0	0
2013	108	15[B]	13.89	0	0
Total	8661	1390	16.05	15	0.2

A/B $p < 0.001$; NHPH – non-*Helicobacter pylori* helicobacters.

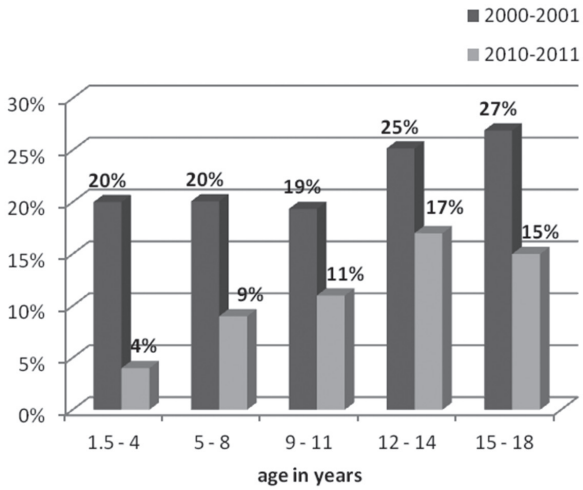

Fig. 1. Changes in the prevalence of *H. pylori* infection in different age groups in the periods 2000–2001 and 2010–2011

between the analyzed periods in these groups were not statistically significant. The prevalence of infection in both periods increased with age and was highest in patients aged 12–18 years (Fig. 1).

The drug sensitivity of 989 *H. pylori* strains to antibiotics, including 734 strains from the years 2000–2004 and 255 strains from the years 2009––2013, was analyzed. In 2000–2004, the resistance rates of *H. pylori* strains to metronidazole and clarithromycin were 48.5% (356/734) and 21.2% (156/734) respectively, while in the years 2009––2013, resistance rates to these antibiotics were 36% (92/255) and 26% (67/255) respectively. In the analyzed periods, detected an increased number of strains resistant to both MZ and CH was detected, from 7.9% (58/734) to about 22.7% (58/255). No strains resistant to amoxicillin were detected (Fig. 2).

Discussion

Recent studies carried out in many countries at both high and low levels of socioeconomic status have reported a decline in the prevalence of *H. pylori* infection, which is primarily due to improvement in the standards of living and sanitary conditions [15, 16]. In developing countries, including Poland, the prevalence of *H. pylori* infec-

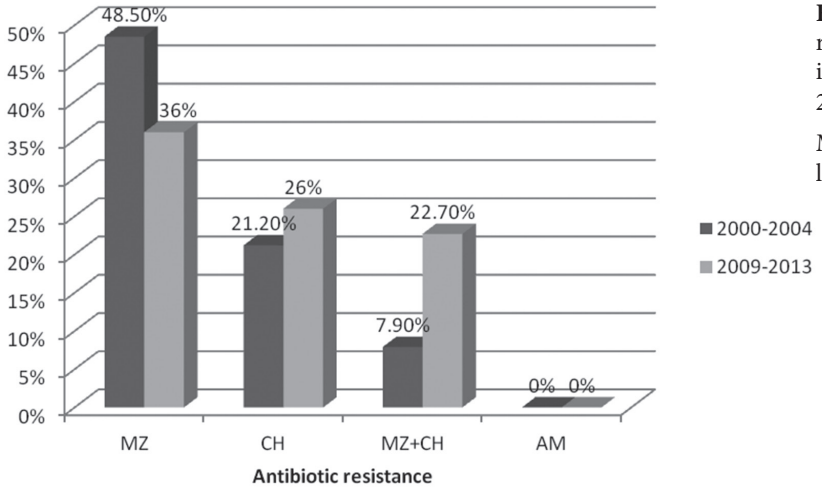

Fig. 2. Comparison of the resistance rates of *H. pylori* strains to antibiotics in the periods 2000–2004 and 2009–2013

MZ – metronidazole; AM – amoxicillin; CH – clarithromycin.

tion is, however, still high. A high rate of infection in adults was observed in the years 2009–2012 in six Latin American countries: Chile, Columbia, Costa Rica, Honduras, Mexico and Nicaragua (75––83%), without a drop in the prevalence of *H. pylori* infection in young people [17]. In Mexico City, among children aged 6–23 years, the incidence of *H. pylori* infection in the period from 2005 to 2010 was 38% [18]. In Europe, a much lower incidence of infection in children aged 3–15 years was reported: Czech Republic 4.5%, Sardinia, Italy 13.3% and Rome 8.7% [18, 19]. In Poland, the epidemiological data are based mainly on a multicenter study of 3435 children conducted in 2002. It was found that the average rate of *H. pylori* infections in Polish children was 34.5% [4]. Data from the year 2008 from the region of Silesia showed a drop in the rate of *H. pylori* infection in healthy children (15.7%) [20, 21]. In that study, the average prevalence of *H. pylori* infection in children with clinical symptoms was similar to observations made in children with abdominal pain in the Pomerania region (16.5%) [22], and were consistent with the observations of other authors in Central and Eastern Europe [23, 24]. In the opinion of the present authors, the observed decline in the prevalence of *H. pylori* infection in children with clinical symptoms might be influenced primarily by better diagnosis of these infections and widespread use of eradication therapy. The number of endoscopic procedures has increased in recent years, and extension of the indications for these procedures, as well as the smaller number of patients in recent years, may have contributed to the results of the present study. Changes in life style, improving economic conditions and hygiene standards may also have an impact on the decline in the incidence of infections caused by these microorganisms. However, further studies to assess this subject are needed, especially in the population of healthy children. The incidence of NHP H infection in the present study was low (0.2%). Similar rates were observed in Bulgarian children (0.3%) and the Italian population (0.1%) [24, 25]. *H. heilmannii* is common in many species of mammals, such as dogs, cats, pigs and rats. In humans, these microorganisms are detected very rarely, but they can lead to significant pathologies in the gastrointestinal tract [26]. The infection probably possesses zoonotic origin, as confirmed by the present authors' study of a child with a pet dog and cat living in a rural area [27].

In the present study, the prevalence of *H. pylori* infection was found to be decreased in all age groups. There were no differences in the prevalence of infection between boys and girls. The largest decrease in the incidence of infection was observed in the youngest children in the two analyzed intervals, which may result from better monitoring for *H. pylori* and the introduction of therapeutic standards in this group of patients.

Resistance to clarithromycin and nitroimidazole is the most frequently observed resistance in *H. pylori* strains. In the present study, the resistance of *H. pylori* strains to metronidazole in the first analyzed period (2000–2004) was 48.5%; in the second analyzed period (2009–2013) it dropped to 36%. In the years 2000–2004 in Europe and the United States, resistance to metronidazole in *H. pylori* strains ranged from 20% to 40% [28, 29]. In the same period, in south-east Asia and Egypt, 100% resistant strains were reported. In the years 1999–2009, a very high prevalence of resistance to metronidazole was reported (70–90%) in Senegal, India, China, Iran, Saudi Arabia, Kuwait and Colombia; while in Japan, resistance to metronidazole was very rare (9–12%) [29]. In 2008–2009, the percentage of *H. pylori* strains resistant to metronidazole among children in Europe was lower (25.7%). In the countries of northern and southern Europe, resistance to metronidazole was comparable (28.6% to 29.7%), while in the countries of Central and Eastern Europe the proportion of resistant strains was significantly higher (43.8%). Differences in the resistance rates to metronidazole may be due to the widespread use of this drug for the treatment of parasitic diseases in developing countries, and for treatment of gynecological and dental infections in developed countries [29, 30].

Resistance to clarithromycin has increased in recent years, which contributes to the failure of first-line therapy in approximately 70% of patients infected with *H. pylori* [30]. In the US, about 12% of strains were resistant to clarithromycin; in Mexico even more (25%); while in 1998, the rate of resistance to clarithromycin in Europe was approximately 9.8% [28]. In the last 10 years, the percentage of *H. pylori* strains resistant to clarithromycin among adults in Europe increased to 17.5%, whereas among children it was twice as high (> 30%) [29, 30]. The present study confirms those authors' observations: The percentage of strains resistant to clarithromycin also increased from 21.2% to 26% in the studied periods. Common use of clarithromycin in respiratory tract infections in children in Poland may also be responsible for the high resistance rates among *H. pylori* strains. Moreover, the present study found an increased number of strains with primary resistance to both clarithromycin and metronidazole, which may also contribute to treatment failure.

In Europe, in the years 2008–2009, the prevalence of *H. pylori* strains resistant to amoxicillin among adults and children was 0.7% and 0.3%, respectively [30]. In Poland, *H. pylori* strains resis-

tant to amoxicillin have not yet been detected, and this was confirmed by previous and current observations [12, 31].

The authors have concluded that *H. pylori* infection is still a common cause of symptoms of the upper gastrointestinal tract in children in the authors' center. There was a decline in the prevalence of *H. pylori* infection in symptomatic children during the analyzed period. The analysis in this study was retrospective in nature; furthermore, it was conducted on strains isolated from children from only one clinical center. Another major limitation of the study was the bias in the selection of subjects. Multicenter studies are needed to show the actual incidence of infection in healthy and symptomatic children.

NHPH infection is a rare cause of gastric pathology. The prevalence of *H. pylori* infection increases with age and is highest in children 12–18 years old. Primary antibiotic resistance of *H. pylori* increased over the study period, but primary resistance to metronidazole declined.

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Address for correspondence:

Monika Biernat
Department of Microbiology
Wroclaw Medical University
Chałubińskiego 4
50-368 Wrocław
Poland
E-mail: mobiernat@gmail.com
Tel.: +48 71 784 12 86

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