

Antimicrobial susceptibility of *Helicobacter pylori* isolates from Lower Silesia, Poland

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Abstract

Introduction: In recent years the failure of standard therapy for *Helicobacter pylori* infections has been observed, which results primarily from the increasing resistance of *H. pylori* strains to antibiotics. The aim of the study was to estimate the prevalence of antimicrobial resistance of *H. pylori* strains isolated from adult symptomatic patients with primary infection in the Lower Silesia Region in Poland.

Material and methods: One hundred and seventy-eight adults aged 19–89 years with dyspeptic symptoms suggesting gastroduodenal pathology were enrolled in the study. The study was performed in the years 2008–2011. Fifty *H. pylori* strains were isolated from gastric biopsy samples of examined patients. Antimicrobial susceptibility to 6 drugs (amoxicillin (AM), clarithromycin (CH), metronidazole (MZ), tetracycline (TC), levofloxacin (LEV), and rifabutin (RB)) was tested by the gradient-diffusion method (E-test method).

Results: The incidence of *H. pylori* infection among examined patients was 35%. From 50 isolated *H. pylori* strains, 24% showed resistance to CH, 42% to MZ and 8% to LEV alone. Multidrug resistance was detected in 26% of strains, whereas 20% of isolates were resistant to MZ and CH. Examined strains were fully susceptible to AM, TC and RB.

Conclusions: Resistance to clarithromycin strains isolated from adults of the Lower Silesia Region in Poland is high and is almost always associated with resistance to metronidazole (CH + MZ). It is necessary to continuously monitor *H. pylori* resistance to drugs used in therapy, especially to clarithromycin. Verification of the existing recommendations of eradication therapy is also needed.

Key words: *Helicobacter pylori*, resistance, symptomatic patients.

Introduction

Resistance of bacterial strains to antibiotics has recently become a major problem in treatment of human infectious diseases. This issue also applies to treatment of *Helicobacter pylori* (*H. pylori*) infection, which remains the main cause of both duodenal and peptic ulcers and is associated with the risk of developing gastric cancer. In recent years the possible role of this infection in extragastric diseases, such as cardiovascular diseases, hema-

tologic diseases, eye and skin diseases, diabetes mellitus, and neurological disorders, has been postulated [1, 2]. So far antibiotics used for eradication of this bacterium include β -lactams, macrolides, tetracyclines and fluoroquinolones. Recommendations of the European Helicobacter Study Group included in the Maastricht III Consensus Report and guidelines of the Polish Society of Gastroenterology advise in first line treatment using two of three antibiotics (amoxicillin, clarithromycin or metronidazole) and a proton pump inhibitor (PPI). Use of amoxicillin with clarithromycin or metronidazole can be administered when *H. pylori* resistance to clarithromycin in the population is lower than 20% and to metronidazole is lower than 40% [3, 4]. The high effectiveness of the above-mentioned regimens until recently ensured eradication of *H. pylori* in most patients, but during the last few years failure of standard eradication therapy has been seen more often, due mostly to increasing resistance of *Helicobacter* strains to antibiotics. The factors affecting *Helicobacter* resistance to antibiotics are world region, age, gender and ethnic background [5]. In Poland, multicenter studies of *Helicobacter* strains' resistance to antibiotics were performed 10 years ago [6]. Because of difficult isolation of bacteria, the evaluation of drug sensitivity is performed most often after eradication failure. Taking into account the above issues, *H. pylori* resistance to antibiotics in the Lower Silesian Region is difficult to estimate.

The aim of the study was to evaluate the primary resistance of *Helicobacter* strains isolated from adults to amoxicillin, clarithromycin, metronidazole, tetracycline, levofloxacin and rifabutin.

Material and methods

The study was performed on strains isolated from adult patients of the Lower Silesia Region in Poland in the years 2008–2011. The study was part of a multicenter project on surveillance of *H. pylori* resistance in different European countries conducted in the years 2008–2010 [7]. Our study involved 178 patients who underwent endoscopic examination of the upper gastrointestinal tract due to complaints from the upper gastrointestinal tract, such as nausea, vomiting, or abdominal pain, suggesting the presence of pathology. Patients had not been 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. The patients had the following conditions: duodenal ulcer ($n = 7$), chronic gastritis

($n = 114$), chronic gastritis and duodenitis ($n = 18$), gastroesophageal reflux disease (GERD) ($n = 15$), and other diseases ($n = 24$), including hiatal hernia and polyps. 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 2 to 3 h in a microbiological laboratory. The gastric biopsies were examined by direct Gram stain and placed on the following media: Columbia agar medium (Difco) with horse blood (7%) and selective supplement (Dent), containing vancomycin 10 mg/l, trimethoprim 10 mg/l, cefsulodin 5 mg/l and amphotericin B 5 mg/l. The cultures were incubated for 3 days at 37°C under microaerophilic conditions. The strains were identified as *H. pylori* by Gram stain morphology, positive culture and positive catalase, oxidase and urease tests. After the primary isolation and identification, the strains were kept frozen at –70°C in Brucella broth containing 15% glycerol. Then the drug sensitivity was determined by gradient diffusion (E-test, BioMerieux) to six antibiotics: amoxicillin (AM), clarithromycin (CH), metronidazole (MZ), tetracycline (TC), levofloxacin (LEV) and rifabutin (RB) by the method described by Glupczynski *et al.* [8]. Microorganisms obtained during the 72-hour culture were suspended in brain-heart infusion broth (BHI) with a density of 3 on the McFarland scale, 10^8 cells (CFU/ml). In the next stages the bacterial suspension was inoculated on Mueller-Hinton medium (Becton Dickinson) supplemented with 10% horse blood. Then E-test antibiotic impregnated with a gradient of concentration was imposed, to determine the value of the minimum inhibitory concentration (MIC) for the growth of bacteria. Incubation was carried out for 72 h at 37°C in a microaerophilic atmosphere. At the end of incubation, the reading was based on the size of the zone of inhibition of *H. pylori* bacilli in the form of an ellipse. Criteria for interpretation of results: MIC ($\mu\text{g/ml}$) for resistant strains: amoxicillin > 0.5, clarithromycin > 1, metronidazole > 8, tetracycline > 1, levofloxacin > 0.5 and rifabutin > 1 [8, 9].

Statistical analysis

Statistical analysis was performed by χ^2 test with or without Yates' correction and χ^2 test among age groups. A p value < 0.05 was considered significant for all tests.

Results

A total of 178 patients aged 18–89 years were included. The patients' age, gender and clinical diag-

nosis are presented in Table I. Among all patients, *H. pylori* infection was found in 62 (34.83%) individuals. *Helicobacter pylori* was isolated in 50 cases and the antibiotic sensitivity was determined. In 3 cases the culture was not successful despite positive direct Gram stain. In another 9 cases evaluation of drug sensitivity was impossible despite positive culture. Among 50 evaluated isolated *H. pylori* strains 12 (24%) showed resistance to clarithromycin, 21 (42%) to metronidazole and 4 (8%) to levofloxacin (Table II). Resistance to clarithromycin and metronidazole was found more often than to amoxicillin, tetracycline, levofloxacin and rifabutin ($p < 0.05$). Different sensitivity to clarithromycin and metronidazole, depending on the age of the patient, has been demonstrated (Table III). The highest clarithromycin resistance (30%) was found in young individuals, whereas the resistance to metronidazole increased with age. The multidrug resistant strains (for two or more antibiotics) were most often isolated from the youngest patients or for patients above 65 years of age, though the differences were not statistically significant ($p > 0.05$). Multidrug resistant strains were isolated from thirteen patients, mostly women with gastritis (Table IV). Among these 13 (26%) strains, 10 (20%) were resistant to clarithromycin and metronidazole, 2 (4%) to metronidazole and levofloxacin, and 1 (2%) strain was resistant to 3 antibiotics: clarithromycin, metronidazole and levofloxacin. Most metronidazole resistant isolates were found in a low drug concentration (256 µg/ml).

Table I. Characteristics of examined patients

Parameter	Number	Percentage
Adults	178	100
Sex		
Women	110	62
Men	68	38
Gender [years]		
19–44	61	34
45–64	69	39
> 65	48	27
Clinical diagnosis		
Duodenal ulcer	7	4
Chronic gastritis	114	64
Chronic gastritis and duodenitis	18	10
GERD	15	8
Other diseases*	24	14

*Hiatal hernia, polyps

Discussion

Knowledge of local antibiotic sensitivity is one of the most important keys to therapy success. Comparing the results of our study to *H. pylori* drug resistance evaluated 10 years ago in Poland and the Lower Silesia Region, we found a considerable increase of *H. pylori* resistance to clarithromycin and metronidazole [6, 10]. In our region,

Table II. The primary resistance and MIC values for 50 isolated strains

Antibiotic	Resistant strains				Range
	Number	% (95% confidence interval)	MIC ₅₀	MIC ₉₀	
CH	12	24 (16.9–31.0)	0.023	32	0.016–256
MZ	21	42 (25.3–58.7)	256	256	0.016–256
LEV	4	8 (4.9–15.0)	0.125	3	0.002–32
AM	0	0 (0.0–0.0)	0.016	0.08	0.016–256
RB	0	0 (0.0–0.0)	0.002	0.02	0.002–32
TC	0	0 (0.0–0.01)	0.047	0.125	0.016–256

CH – clarithromycin, MZ – metronidazole, LEV – levofloxacin, AM – amoxicillin, RB – rifabutin, TC – tetracycline, MIC₅₀ – minimal inhibitory concentration required to inhibit the growth of 50% of organisms, MIC₉₀ – minimal inhibitory concentration required to inhibit the growth of 90% of organisms

Table III. Distribution of primary resistance of examined *H. pylori* strains in different age groups

Age group	Number of <i>H. pylori</i> strains	Isolates with resistance						Multidrug resistant isolates					
		CH		MZ		LEV		MZ + CH		MZ + CH + LEV		MZ + LEV	
		n	%	n	%	n	%	n	%	n	%	n	%
19–44	20	6	30	5	25	2	10	4	20	1	5	1	5
45–64	19	3	16	11	58	1	5	3	16	0	0	1	5
> 65	11	3	27	5	45	1	9	3	27	0	0	0	0
Total	50	12	24	21	42	4	8	10	20	1	2	2	4

Table IV. Group of patients from whom multi-resistant *H. pylori* strains were isolated

No.	Age	Gender	Diagnosis	MIC [$\mu\text{g/ml}$]					
				CH	MZ	AM	TC	LEV	RB
1	25	F	Gastritis	8	> 256	0.047	< 0.016	32	< 0.002
2	25	F	Gastritis	6	> 256	0.016	0.094	0.125	< 0.002
3	25	F	Gastritis	32	256	0.008	< 0.016	0.125	0.002
4	27	F	Gastritis	< 0.016	> 256	0.016	0.047	> 256	< 0.002
5	28	M	Gastritis	32	> 256	0.023	0.125	0.125	< 0.002
6	32	F	Gastritis	4	256	< 0.002	< 0.016	0.032	< 0.002
7	53	M	Gastritis	256	256	0.002	0.047	0.064	< 0.002
8	55	F	Gastritis	3	8	0.006	0.016	0.032	< 0.002
9	57	F	Gastritis	0.023	128	0.016	0.047	3	< 0.002
10	60	F	Gastritis, hernia	12	> 256	0.016	0.032	0.064	< 0.002
11	68	F	Gastritis	32	> 256	0.064	0.047	0.125	< 0.002
12	74	M	Gastritis	6	256	0.016	0.047	0.125	< 0.002
13	76	M	Gastritis, hernia	8	> 256	0.016	0.064	0.19	< 0.002

F – female, M – male

resistance to clarithromycin in adults is high (24%; formerly 15%). A stable increasing trend of *H. pylori* resistance to clarithromycin is recently being observed; globally 5–28% of *H. pylori* strains are clarithromycin resistant. Based on multicenter studies performed in Europe, notable differences in clarithromycin resistance were found among different European countries (5–20%) [11–13]. In our study, we found metronidazole resistance in up to 42% of patients deriving from our region, compared to 27–52% formerly [6, 14]. Resistance of *H. pylori* to metronidazole varies from 20% to 80%, for instance, in Japan 1–12%, USA 20–40%, Europe 50–80%, Mexico 70–80%, Africa 80% [11–13]. Up to now in Poland there have been no amoxicillin or tetracycline resistant strains found, as was also confirmed in our study [6]. In other countries, resistance to these two antibiotics is constantly increasing: for amoxicillin in Bulgaria 2.4%, Spain 1.4%; and for tetracycline in Sweden 0.3%, Great Britain 0.5%, Korea 8.8% [13, 15, 16]. The analysis performed in several European centers proved high efficacy of levofloxacin, one of the new fluoroquinolones, in combination with amoxicillin and a proton pump inhibitor for *Helicobacter* eradication therapy. This treatment could be a good alternative for standard therapy failure [17–19]. Fast growth of resistance to fluoroquinolones used commonly for other indications should also be considered. In our study we observed slight *H. pylori* resistance to levofloxacin, though it was not previously used in the eradication therapy. Rifabutin could be another possible alternative for unsuccessful therapy, but it can be used after several treatment failures [20].

In our study 20% of *H. pylori* isolates were resistant to metronidazole and clarithromycin together. The double resistance to these antibiotics in Europe is low (0.6–8.9%), while in Asia the level is 8.3% [11, 13]. Common use of clarithromycin in upper respiratory tract infections and metronidazole in gynecological and parasitic infections can considerably increase the *H. pylori* resistance to these two agents [5]. This theory can explain the high incidence of clarithromycin-resistant *H. pylori* strains and multi-drug resistance in young adults, especially in women. In Poland antibiotic abuse, especially macrolides in upper respiratory tract infections, is common [21]. Some authors report that *Helicobacter* eradication failure is not only caused by antibiotic resistance but also may depend on other bacterial or host factors [22].

The coincidence of resistance to levofloxacin with clarithromycin and/or metronidazole resistance might also be distressing. It seems that the frequency of such multidrug resistant *Helicobacter* isolates will increase in the future.

This study has some limitations. The analysis included only 50 strains. It is necessary to conduct studies on a larger number of *H. pylori* strains from different regions of the country, so as to know the exact scale of the problem of primary resistance. Furthermore, the strains derived from patients with varying clinical diagnosis, especially with chronic gastritis. In the group studied, there was a small percentage of patients with peptic ulcer disease, in which the profile of phenotypic susceptibility of *H. pylori* strains may vary. In conclusion, we found in our study that resistance of *H. pylori* isolates from adults in the Lower Silesia Region to clarithromycin

is high and almost always connected with resistance to metronidazole (MZ + CH). Primary resistance to levofloxacin is low, suggesting a possible alternative in case of *H. pylori* eradication failure. Constant monitoring of *H. pylori* resistance to antibiotics used in the therapy is necessary in our region, especially for clarithromycin, as well as verification of guidelines for *Helicobacter* eradication.

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