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Primary and secondary clarithromycin, metronidazole, amoxicillin and levofloxacin resistance to *Helicobacter pylori* in southern Poland

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Abstract:
The aim of this study was to assess the primary and secondary resistance of *H. pylori* strains cultured from adult patients of the Małopolska region of Poland, mainly of Kraków and the surrounding areas, to antibacterial agents (amoxicillin, clarithromycin, metronidazole and levofloxacin). In total, 115 *H. pylori* strains were isolated, of which 90 strains originated from patients who had never been treated for *H. pylori* infection, while the remaining 25 were isolated from patients in whom eradication of the infection failed after treatment. All tested *H. pylori* strains were susceptible to amoxicillin. Forty-four percent of strains isolated were resistant to metronidazole. The primary and secondary resistance to this antimicrobial chemotherapeutic reached 37% and 72% ($p = 0.002$), respectively. In total, 34% of strains were resistant to clarithromycin, and the ratio of strains with secondary resistance was significantly greater than that of the strains with primary resistance (80% *vs.* 21%, $p < 0.001$). The double resistance to both metronidazole and clarithromycin was confirmed in 23% of *H. pylori* strains. Five percent of *H. pylori* strains were resistant to levofloxacin, while primary and secondary resistance to this drug accounted for 2% and 16% ($p = 0.006$), respectively. In total, 4% of *H. pylori* strains were simultaneously resistant to metronidazole, clarithromycin and levofloxacin. Thus, the high resistance to metronidazole and clarithromycin excludes the possibility of using these drugs for treatment of *H. pylori* infection without earlier antibiogramming. Levofloxacin, as a drug of high efficacy against *H. pylori*, should be reserved for an "emergency" therapy and used in a limited capacity in order to preserve its potent antimicrobial activity. The Polish Society of Gastroenterology recommends levofloxacin as a third-line therapy [14].

Key words:
Helicobacter pylori, resistance, amoxicillin, clarithromycin, metronidazole, levofloxacin

Abbreviations: AMX – amoxicillin, CL – clarithromycin, LE – levofloxacin, MALT – Mucosa-Associated Lymphoid Tissue, MIC – minimum inhibitory concentration, MTZ – metronidazole, NS – not significant, PPI – proton pump inhibitor

Introduction

Helicobacter pylori (*H. pylori*) plays a significant role in the pathogenesis of upper alimentary tract diseases. The organism is an etiological factor of type B gastritis and is one of the essential risk factors in peptic ulcer disease, gastric Mucosa-Associated Lymphoid Tissue (MALT), lymphoma, and gastric carcinoma [1, 11, 13, 21, 25, 26, 35, 36]. The role of *H. pylori* in extraintestinal diseases has also been suggested [14, 33, 34].

The discovery and isolation of *H. pylori* occurred in 1982 by Robin Warren and Barry Marshall. Subsequently demonstrating its involvement in the abovementioned diseases marked a turning point in gastroenterology and contributed to the use of antibacterial agents to treat these diseases [16, 29].

In 10–15% of *H. pylori* cases, gastric or duodenal ulcers are developed, while in 2% of them, neoplastic changes can occur. The risk of gastric neoplasm increases with prolonged duration of infection [25, 26, 35]. Early introduction of a proper therapy according to the European Helicobacter Study Group (EHSG) guidelines results in eradication of the infection, healing of the ulcers, reduction in relapses, and decreases in the intensity of mucosal infection. Consequently, the risk of neoplastic changes is also reduced [26, 28, 35].

At present, in Poland, the obligatory procedures for management of *H. pylori* infection, as elaborated upon by the Working Group of the Polish Society of Gastroenterology (Consensus 2008), are based on new guidelines of EHSG (Maastricht III 2005) [14, 28].

Despite the introduction of combination therapy, including either a proton pump inhibitor PPI with two antibiotics (amoxicillin, clarithromycin and rarely tetracycline) or an antibiotic with one chemotherapeutic (metronidazole or tynidazole), the eradication of *H. pylori* is not always complete. The growing resistance of clinical *H. pylori* strains to antibacterial drugs represents one of the major factors responsible for the failure of eradication [2, 31]. This is why the Maastricht III Consensus Report recommends permanent local monitoring of *H. pylori* resistance to antibiotics to assess the risk of failure of recommended empirical therapy and also to choose the appropriate antibacterial treatment [28].

The abundance of metronidazole- and clarithromycin-resistant *H. pylori* strains in Poland and around the world serves as a stimulus to investigate alternative combination therapies [9, 15, 27, 31]. Zullo studies and other have shown, that effective therapy consists of a sequential therapy regimen [12, 17, 39]. The therapy is based on administration of two drugs, PPI and amoxicillin, for the first 5 days, and of three drugs, PPI, clarithromycin, and tynidazole, for the following 5 days. These studies confirmed the eradication of *H. pylori* in 95% of treated patients [39].

Implementation of this treatment strategy may be of high importance in the eradication of *H. pylori* infection in children, for whom the drugs recommended as emergency therapy for adults, tetracycline and quinolone, are contraindicated.

The aim of this study was to assess the primary and secondary resistance of *H. pylori* strains isolated from adult patients from the Małopolska region of Poland, mainly from Kraków and surrounding areas, to antibacterial drugs (amoxicillin, metronidazole, clarithromycin and levofloxacin) used clinically for eradication.

Materials and Methods

Patients, clinical material

This study enrolled a cohort of 115 patients who presented with dyspepsia and sought care at the Gastrological Outpatient Clinic of the Center of Medical Care "Falck Medycyna" in Kraków between December 2006 and December 2008. Ninety of the patients had never been treated for *H. pylori* infection, while the other 25 patients were treated and failed to improve during therapy.

The patients underwent gastroscopy, during which specimens from changed gastric mucosa of the antrum and the body regions were biopsied to culture *H. pylori* and test drug susceptibility. The bioptates were transferred to a transportation medium, Portagerm pylori (PORT-PYL, bioMérieux, Marcy-l'Etoile, France), and were then sent to the Department of Pharmaceutical Microbiology of the Jagiellonian University Medical College.

The plan for the study was approved by the Bioethical Commission of Jagiellonian University, and every patient consented in writing to participate in the trial.

H. pylori culture and drug-susceptibility testing

Bioptates of gastric mucosa collected from gastroscopy were homogenized in glass sterile mortars and inoculated onto Schaedler Agar + 5% sheep blood (bioMérieux, Marcy-l'Etoile, France, Oxoid, Basingstoke, UK) with and without DENT supplementation (Helicobacter pylori Selective Supplement – DENT, Oxoid, Basingstoke, UK). The culture was conducted for 3 to 7 days under microaerophilic conditions at 37°C.

The cultured strains were identified by the macroscopic appearance of colonies and positive tests for urease, catalase and oxidase. Additionally, Gram-staining of the culture was conducted to verify the presence of Gram-negative spiral bacteria.

The susceptibility of *H. pylori* strains to amoxicillin, metronidazole, clarithromycin and levofloxacin was assessed quantitatively by E-test method (AB Biodisk, Solna, Sweden), which establishes the lowest concentration of the drug that inhibits the growth of the infectious organism (minimum inhibitory concentration – MIC).

E-test, according to the Technical Information (AB Biodisk, Etest Technical Manual) provided, is a quantitative technique for determining the antimicrobial susceptibility of Gram-negative and Gram-positive aerobic bacteria and fastidious bacteria, such as *H. pylori*. The system comprises a predefined antibiotic gradient that is used to determine the MIC, in mg/l, of different antimicrobial agents against microorganisms as tested on agar media using overnight incubation.

E-test is a thin, inert and non-porous plastic strip. One side of the strip carries the MIC reading scale in mg/l and a two or three-letter code on the handle to designate the identity of the antibiotic. A predefined exponential gradient of the antibiotic, dried and stabilized, is immobilized on the opposite surface of the strip.

When an E-test gradient strip is applied to an inoculated agar surface, there is an immediate and effective transfer of the preformed antibiotic gradient on the plastic carrier surface into the agar matrix. A stable, continuous and exponential gradient of antibiotic concentrations is formed directly underneath the strip. After incubation, whereby bacterial growth becomes visible, a symmetrical inhibition ellipse centered along the strip can be seen. The MIC value is read from the scale in terms of mg/l where the ellipse edge intersects the strip (Figs. 1, 2).

Pure *H. pylori* culture grown for 72 h in 0.85% NaCl (API 0.85% NaCl Medium, bioMérieux, Marcy-l'Etoile, France) was prepared. The density of the suspension as measured by the densimeter (bioMérieux,

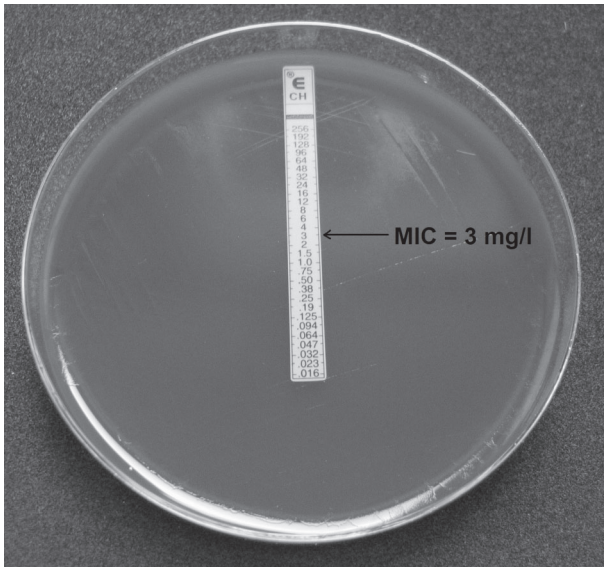

Fig. 1. Susceptibility of *H. pylori* clinical strain to clarithromycin as tested by E-test; an example of a resistant isolate with MIC value = 3 mg/l (MIC ≥ 1 mg/l for resistant strains)

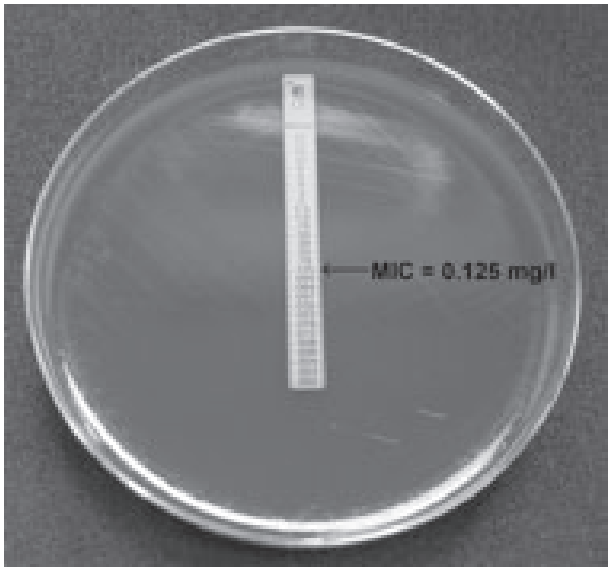

Fig. 2. Susceptibility of *H. pylori* clinical strain to levofloxacin as tested by E-test, an example of a susceptible isolate with MIC value = 0.125 mg/l (MIC > 1 mg/l for resistant strains)

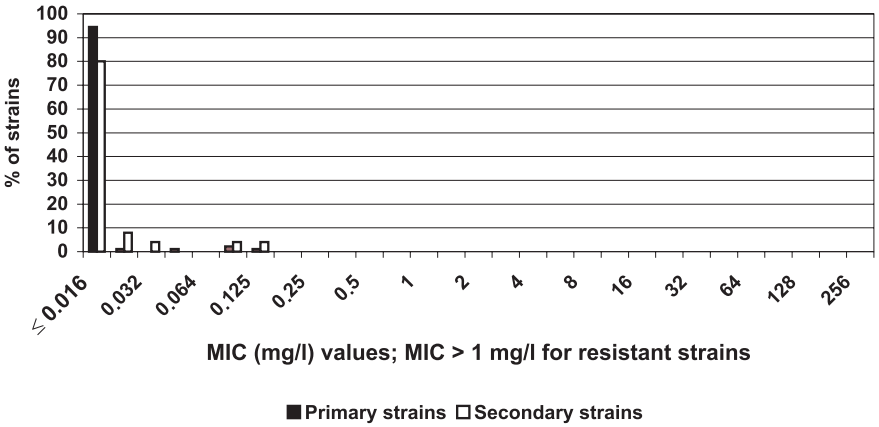

Fig. 3. Activity of amoxicillin against primary and secondary *H. pylori* strains expressed by MIC values (MIC > 1 mg/l for resistant strains). *H. pylori* strains were defined as susceptible when the MICs of amoxicillin were between 0.016 mg/l and 1 mg/l. Strains resistant to amoxicillin were not detected (MIC values > 1 mg/l). Non-significant difference in resistance to AMX between primary and secondary strains (p > 0.05)

Marcy-l'Etoile, France) was equivalent to 3.0 McFarland units (approximately 10^8 CFU/ml). A sterile swab brush was placed into the suspension and then a Schaedler Agar with 5% sheep blood plate was inoculated followed by an E-test. The culture was grown for 72 h under microaerophilic conditions at 37°C. Afterwards, the MIC values were recorded.

Resistant strains were defined as those with the following MIC values: amoxicillin > 1 mg/l, metronidazole > 4 mg/l, clarithromycin > 1 mg/l and levofloxacin > 1 mg/l [3, 7, 10].

Statistical analysis

The statistical analysis was conducted with the use of a χ^2 test. Value of α was set at 0.05 for the analysis.

Results

A total of 115 *H. pylori* strains were isolated, of which 90 originated from patients who had never been treated for *H. pylori* infection (primary strains) and 25 originated from patients where eradication failed (secondary strains).

All *H. pylori* strains tested were susceptible to amoxicillin (Tab. 1). The growth of 95% of primary strains and 80% of secondary strains was inhibited at MIC ≤ 0.016 mg/l. Somewhat higher MIC values, but still in the range of susceptibility to amoxicillin, were noted for only 5% of primary isolates and for as many as 20% of secondary isolates (Fig. 3).

Tab. 1. Comparison of primary and secondary resistance to amoxicillin, metronidazole, clarithromycin and levofloxacin of 115 clinical isolates of *H. pylori*

Antimicrobial agent	No. (%) of resistant strains			p
	All strains	Primary strains	Secondary strains	
	(n = 115)	(n = 90)	(n = 25)	
MTZ	51 (44)	33 (37)	18 (72)	0.002*
CL	39 (34)	19 (21)	20 (80)	< 0.001*
LE	6 (5)	2 (2)	4 (16)	0.006*
AMX	0	0	0	NS

MTZ – metronidazole, CL – clarithromycin, LE – levofloxacin, AMX – amoxicillin; p (χ^2 test), the value p ≤ 0.05 was deemed statistically significant; * statistically significant differences in resistance to MTZ, CL and LE between primary and secondary strains; NS – non-significant difference in resistance to AMX between primary and secondary strains

The percentage of *H. pylori* strains resistant to metronidazole accounted for 44%. The primary and secondary resistance amounted to 37% and 72% (p = 0.002), respectively (Tab. 1). High resistance to metronidazole (MIC ≥ 256 mg/l) was found in 20% and 44% of *H. pylori* strains isolated from patients before and after treatment, respectively (Fig. 4).

In total, 34% of *H. pylori* strains were resistant to clarithromycin and the ratio of the strains with secondary resistance was significantly greater as compared to those with primary resistance (80% *vs.* 21%, p <

0.001) (Tab. 1). The MIC ≤ 0.016 mg/l was noted for 74% of primary strains and only for 20% of secondary strains. For the rest of the strains, the MIC values were between 0.38 and 64 mg/l, which corresponds to an intermediate susceptibility or resistance (Fig. 5).

A double resistance to both metronidazole and clarithromycin was confirmed in 23% (27/115) of *H. pylori* strains. For these drugs, the percentages of primary and secondary resistant strains accounted for 13% (12/90) and 60% (15/25), respectively.

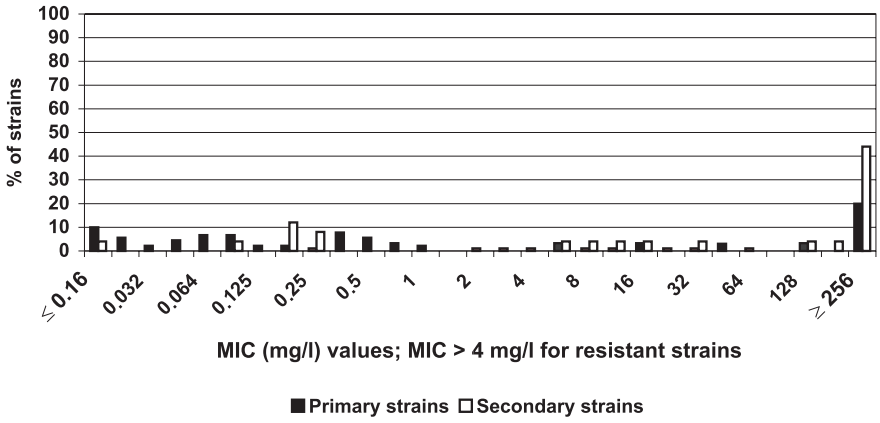

Fig. 4. Activity of metronidazole against primary and secondary *H. pylori* strains expressed by MIC values (MIC > 4 mg/l for resistant strains). *H. pylori* strains were defined as susceptible when the MICs of metronidazole were between 0.016 mg/l and 4 mg/l. MIC values for resistant strains were more than 4 mg/l to 256 mg/l. Statistically significant difference in resistance to MTZ between primary and secondary strains ($p \leq 0.05$)

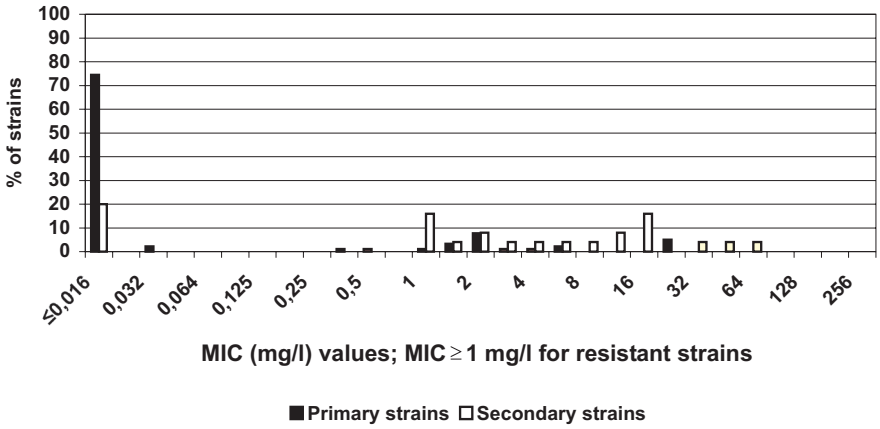

Fig. 5. Activity of clarithromycin against primary and secondary *H. pylori* strains expressed by MIC values (MIC ≥ 1 mg/l for resistant strains). *H. pylori* strains were defined as susceptible when the MICs of clarithromycin were between 0.016 mg/l and 0.25 mg/l. MIC value for intermediate susceptible strains was 0.5 mg/l. MIC values for resistant strains were between 1 mg/l and 256 mg/l. Statistically significant difference in resistance to CL between primary and secondary strains ($p \leq 0.05$)

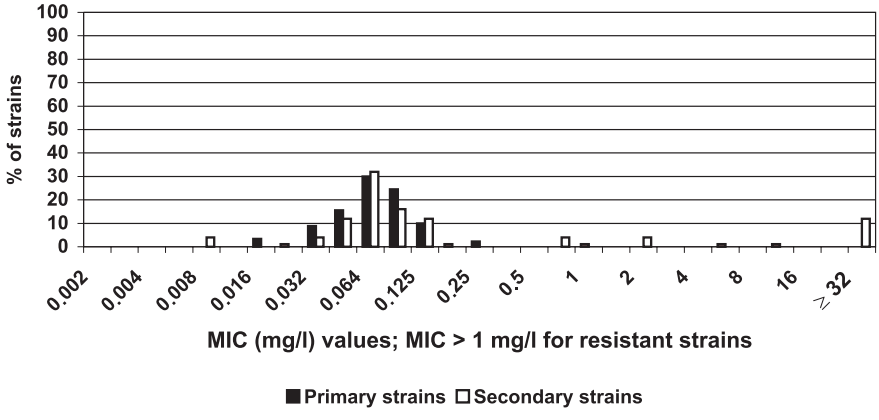

Fig. 6. Activity of levofloxacin against primary and secondary *H. pylori* strains expressed by MIC values (MIC > 1 mg/l for resistant strains). *H. pylori* strains were defined as susceptible when the MICs of levofloxacin were between 0.002 mg/l and 1 mg/l. MIC values for resistant strains were more than 1 mg/l to 32 mg/l. Statistically significant difference in resistance to LE between primary and secondary strains ($p \leq 0.05$)

Five percent of *H. pylori* strains were resistant to levofloxacin and primary and secondary resistance to this drug accounted for 2% and 16% ($p = 0.006$), respectively (Tab. 1). The activity of levofloxacin against the tested strains was reasonably high with a predominance of MIC values ranged between 0.032 mg/l and 0.125 mg/l for 89% of primary isolates and 76% of secondary (Fig. 6).

It is notable that 4% (5/115) of strains showed simultaneous resistance to three drugs: metronidazole, clarithromycin and levofloxacin. The ratio of strains with primary and secondary resistance to these drugs accounted for 1% (1/90) and 16% (4/25), respectively.

Discussion

Susceptibility of a bacterium to antibiotics and chemotherapeutics represents one of the most important factors in determining the potential effectiveness of *H. pylori* infection treatments. It was proven that resistance to clarithromycin and, in a lesser extent, to metronidazole, is responsible for the failure of therapies that include these drugs. In the case of strains susceptible to clarithromycin, the eradication amounted to 87.8%, while in the case of strains resistant to this antibiotic, the eradication dropped to 18.3%. The susceptibility to metronidazole resulted in 97% eradication, while resistance to this chemotherapeutic resulted in a 25% decrease in eradication [31].

Since the 1990s, when clarithromycin was introduced as a therapy that can be used to treat *H. pylori*, a growing number of *H. pylori* clarithromycin-resistant strains have been observed in many countries. This is probably a result of the frequent use of the drug in dental and respiratory system infections [5, 15, 22, 30, 31].

The geographic differences in the percentage of resistant strains can likely be associated with prescribing patterns, administration, and cost. It was demonstrated that the percentage of resistant strains is proportional to the yearly consumption of this antibiotic [5, 24, 31]. The percentages of resistant strains differ also between age groups (children and adults), which probably results from the increased prescription of macrolides notably in children for respiratory tract infections [5, 15, 24, 31].

The study conducted in Kraków between 1999 and 2002 revealed that a total of 48.6% of strains were resistant to clarithromycin, where the ratio of strains with secondary resistance (66.7%) was considerably higher than that of strains isolated from patients before treatment (22.2%) [37]. Somewhat different results were obtained in studies conducted between 2001 and 2004 in other centers in Poland (Warszawa, Płock, Rzeszów, Sanok, Katowice), where a total of 28% of *H. pylori* strains were resistant to clarithromycin [15]. The primary resistance to this antibiotic was almost the same as in the aforementioned Kraków study, accounting for 22% of cases, whereas the percentage of the strains with secondary resistance was lower, at 54% [15]. The results of primary resistance to clarithromycin obtained by centers involved in the studies showed considerable differences from region to region (ranging from 0% to 33%) as well as between adults (15%) and children (28%) [15].

In our study, the percentage of strains primarily resistant to clarithromycin was close to the abovementioned results and accounted for 21%, indicating that primary resistance to this drug has not increased. However, the secondary resistance was considerably higher, at 80%.

Maastricht III Consensus 2005 recommends excluding the use of clarithromycin or, prior to including it as a part of eradication therapy, perform an antibiogram if, in a given population, primary resistance of *H. pylori* strains to clarithromycin is higher than 15–20% [28]. Therefore, according to the above data, in Poland, especially in the Małopolska region, clarithromycin should not be used in the treatment of *H. pylori* infection without a preliminary assessment of drug susceptibility.

As was shown in our analysis of MIC distribution, clarithromycin may still be a highly effective drug, but only in patients infected with susceptible strains.

It was shown that resistance to clarithromycin usually results in the failure of eradication therapy [6]. For that reason, susceptibility to this drug should be determined before every administration.

In many countries, high percentages of *H. pylori* strains that are primarily resistant to metronidazole are reported (South Korea: 66.2%, Mexico: 58%, Brazil: 55%), which probably results from the widespread use of low-cost nitroimidazoles in the treatment of parasitic, dental and gynecological infections [8, 20, 23].

Between 1999 and 2002 in Kraków, a total of 58.5% of strains were noted to be resistant to metronidazole. Primary and secondary resistance was reasonably high and accounted for in 46.7% and 66.7%

of cases, respectively [37]. Comparable results were obtained in Poland-wide studies conducted in the period of 2001–2004, in which the primary resistance to metronidazole accounted for 41% and the secondary for 68% of the instances. The total resistance to this chemotherapeutic reached 46% [15]. Similarly, in our study, the resistance to metronidazole amounted to 44%. Only 37% of strains isolated from patients prior to treatment were resistant to metronidazole. However, the ratio of strains with secondary resistance was somewhat higher and accounted for 72%.

Despite high resistance to metronidazole reported in many countries, Maastricht III Consensus Report 2005 does not recommend a routine determination of *H. pylori* susceptibility to this drug, as the resistance of *H. pylori* to metronidazole *in vitro* does not necessarily correspond with the resistance seen *in vivo*. Moreover, the methods for determination of *H. pylori* susceptibility to this chemotherapeutic agent require more standardization [19, 28].

Due to frequent use of metronidazole and clarithromycin in the treatment of *H. pylori* infection, the strains resistant to both drugs are being found more often [31].

In Poland, according to the studies conducted between 1999 and 2004, the percentage of *H. pylori* strains with double primary resistance to metronidazole and clarithromycin accounted for 15.5% or 13%, depending on the study [15, 37]. Similar results were obtained in our study, which revealed 13% of such strains.

Resistance of *H. pylori* to amoxicillin is very low all over the world and, in most countries, has not been noted [31]. This finding was confirmed by Poland-wide studies conducted in the years 1999 to 2004 as well as by our study, which revealed that all *H. pylori* strains were susceptible to amoxicillin. This situation has not changed for years, despite the frequent use of amoxicillin in *H. pylori* eradication, as well as in the treatment of other infections [15, 37]. The exceptions are Brazil and South Korea, where high ratios of amoxicillin resistant strains were found to be 38% and 18.5%, respectively [20, 23].

As can be seen from our present study, although amoxicillin is still a highly effective antibiotic, the shift in MIC for this drug towards higher values could be of concern. This phenomenon is most apparent in cases of strains that have been isolated from patients after treatment. This means that the emergence of future resistant strains to this drug could result in a drop of effectiveness in *H. pylori* infection treatment.

Recently, new hopes were raised with the introduction of levofloxacin for eradication therapy. Trials have confirmed an improvement in the effectiveness of eradication of *H. pylori* with the use of levofloxacin [18, 32].

However, in some countries, such as South Korea, Italy or Belgium, the percentages of strains primarily resistant to this drug are reasonably high and account for 21.5, 19.1 and 16.8%, respectively [3, 23, 38]. Most likely, these figures reflect high usage of fluoroquinolones in the treatment of various infections.

In our study, a total of 5% of strains were resistant to levofloxacin, but primary resistance was significantly lower in comparison to the above-cited results and accounted for 2% of cases, while the ratio of strains with secondary resistance was 16%. Taking into account a wide distribution range of the levofloxacin MIC values against *H. pylori* strains, one can predict that the ratio of strains resistant to this drug will increase in the future. The use of levofloxacin seems to be justified only in the treatment of serious cases, as resistance to this drug is readily acquired by bacteria [9].

It is especially worth emphasizing that in our study, 4% of *H. pylori* strains were resistant to three out of four tested drugs, which included metronidazole, clarithromycin and levofloxacin. Multi-resistance among *H. pylori* strains was also noted in other countries, such as Italy, Bulgaria, Brazil and South Korea [4, 20, 23, 38]. These findings raise concerns about effective *H. pylori* eradication in the future.

Our study contains some limitations, including missing data on susceptibility of *H. pylori* strains that were present in the group of 25 patients before the failed eradication occurred. Perhaps the very high ratio of strains with secondary resistance to clarithromycin and metronidazole in our study was caused by resistant strains present in some patients prior to treatment.

In summary, a high ratio of *H. pylori* strains resistant to metronidazole and clarithromycin, which are fundamental drugs used in the treatment of *H. pylori* infection, and the emergence of multi-resistant isolates indicate the necessity to replace the first-line empirical treatment with targeted treatment in accordance with a performed antibiogram. This will improve the effectiveness of the treatment and slow the growth of resistance. Levofloxacin should be reserved for emergency therapy only.

References:

1. Ando T, Goto Y, Maeda O, Watanabe O, Ishiguro K, Goto H: Causal role of *Helicobacter pylori* infection in gastric cancer. World J Gastroenterol, 2006, 12, 181–186.
2. Bąk-Romaniszyn L, Płaneta-Małecka I: Reasons for failure in *Helicobacter pylori* eradication. (Polish). Ped Wsp Gastroent Hep Żyw Dziecka, 2004, 6, 387–391.
3. Bogaerts P, Berhin C, Nizet H, Glupczynski Y: Prevalence and mechanisms of resistance to fluoroquinolones in *Helicobacter pylori* strains from patients living in Belgium. Helicobacter, 2006, 11, 441–445.
4. Boyanova L, Gergova G, Nikolov R, Davidkov L, Kamburov V, Jelev C, Mitov I: Prevalence and evolution of *Helicobacter pylori* resistance to 6 antibacterial agents over 12 years and correlation between susceptibility testing methods. Diagn Microbiol Infect Dis, 2008, 60, 409–415.
5. Boyanova L, Mentis A, Gubina M, Rozynek E, Gosciniak G, Kalenic S, Göral V et al.: The status of antimicrobial resistance of *Helicobacter pylori* in eastern Europe. Clin Microbiol Infect, 2002, 8, 388–396.
6. Broutet N, Tchamgoué S, Pereira E, Lamouliatte H, Salamon R, Mégraud F: Risk factors for failure of *Helicobacter pylori* therapy – results of an individual data analysis of 2751 patients. Aliment Pharmacol Ther, 2003, 17, 99–109.
7. BSAC Methods for Antimicrobial Susceptibility Testing, Version 7, 2008.
8. Chihu L, Ayala G, Mohar A, Hernández A, Herrera-Goepfert R, Fierros G, González-Márquez H, Silva J: Antimicrobial resistance and characterization of *Helicobacter pylori* strains isolated from Mexican adults with clinical outcome. J Chemother, 2005, 17, 270–276.
9. Cianci R, Montalto M, Pandol F, Gasbarrini G B, Cammarota G: Third-line rescue therapy for *Helicobacter pylori* infection. World J Gastroenterol, 2006, 12, 2313–2319.
10. CLSI M100-S18, Performance Standards for Antimicrobial Susceptibility Testing, Eighteenth Informational Supplement Clinical and Laboratory Standards Institute, ISBN: 1562386530, 2008.
11. Cremonini F, Gasbarrini A, Armuzzi A, Gasbarrini G: *Helicobacter pylori* – related diseases. Eur J Clin Invest, 2001, 31, 431–437.
12. De Francesco V, Zullo A, Margiotta M, Marangi S, Burattini O, Berloco P, Russo F et al.: Sequential treatment for *Helicobacter pylori* does not share the risk factors of triple therapy failure. Aliment Pharmacol Ther, 2004, 19, 407–414.
13. Dunn BE, Cohen H, Blaser MJ: *Helicobacter pylori*. Clin Microbiol Rev, 1997, 10, 720–741.
14. Dzieniszewski J, Jarosz M; Polish Society of Gastroenterology *Helicobacter pylori* Working Group. Consensus of the Polish Society of Gastroenterology *Helicobacter pylori* Working Group on diagnostic and therapeutic approaches for the *Helicobacter pylori* infection (2008) (Polish). Gastroenterol Pol, 2008, 15, 323–331.
15. Dzierżanowska-Fangrat K, Rożynek E, Celińska-Cedro D, Jarosz M, Pawłowska J, Szadkowski A, Budzyńska A et al.: Antimicrobial resistance of *Helicobacter pylori* in Poland: a multicentre study. Int J Antimicrob Agents, 2005, 26, 230–234.
16. European Helicobacter Pylori Study Group: Current European concepts in the management of *Helicobacter pylori* infection. The Maastricht Consensus Report. Gut, 1997, 41, 8–13.
17. Francavilla R, Lionetti E, Castellaneta S P, Magistà A M, Boscarelli G, Piscitelli D, Amoruso A et al.: Improved efficacy of 10-day sequential treatment for *Helicobacter pylori* eradication in children: a randomized trial. Gastroenterology, 2005, 129, 1414–1419.
18. Gatta L, Zullo A, Perna F, Ricci C, De Francesco V, Tampieri A, Bernabucci V et al.: A 10-day levofloxacin-based triple therapy in patients who have failed two eradication courses. Aliment Pharmacol Ther, 2005, 22, 45–49.
19. Gerrits MM, van der Wouden EJ, Bax DA, van Zwet AA, van Vliet AHM., de Jong A, Kusters JG et al.: Role of the rdxA and frxA genes in oxygen-dependent metronidazole resistance of *Helicobacter pylori*. J Med Microbiol, 2004, 53, 1123–1128.
20. Godoy AP, Ribeiro ML, Benvengo YH, Vitiello L, Miranda M de C, Mendonca S, Pedrazzoli J jr: Analysis of antimicrobial susceptibility and virulence factors in *Helicobacter pylori* clinical isolates. BMC Gastroenterol, 2003, 3, 20.
21. Graham DY: *Helicobacter pylori* infection is the primary cause of gastric cancer. J Gastroenterol, 2000, 35, 90–97.
22. Karczewska E, Wojtas I, Budak A: Occurence of Helicobacter pylori primary resistance to antibacterial drugs in Poland and in the world (Polish). Post Mikrobiol, 2009, 48, 31–41.
23. Kim JM, Kim JS, Kim N, Kim SG, Jung HC, Song IS: Comparison of primary and secondary antimicrobial minimum inhibitory concentrations for *Helicobacter pylori* isolated from Korean patients. Int J Antimicrob Agents, 2006, 28, 6–13.
24. Koletzko S, Richy F, Bontems P, Crone J, Kalach N, Monteiro ML, Gottrand F et al.: Prospective multicentre study on antibiotic resistance of *Helicobacter pylori* strains obtained from children living in Europe. Gut, 2006, 55, 1711–1716.
25. Konturek JW: Discovery by Jaworski of *Helicobacter pylori* and its pathogenetic role in peptic ulcer, gastritis and gastric cancer. J Physiol Pharmacol, 2003, 54, 23–41.
26. Konturek PC, Konturek SJ, Brzozowski T: *Helicobacter pylori* infection in gastric cancerogenesis. J Physiol Pharmacol, 2009, 60, 3–21.
27. Łaszewicz W: Studies on *Helicobacter pylori* infection (Polish). Trans Humana, Białystok, 2004.
28. Malfertheiner P, Megraud F, O' Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R et al.: Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. Gut, 2007, 56, 772–781.
29. Marshall BJ, Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet, 1984, 16, 1311–1315.
30. Megraud F: Antibiotic resistance in *Helicobacter pylori* infection. Br Med Bull, 1998, 54, 207–216.
31. Megraud F: *H. pylori* antibiotic resistance: prevalence, importance, and advances in testing. Gut, 2004, 53,1374–1384.

32. Nista EC, Candelli M, Zocco MA, Cremonini F, Ojetti V, Finizio R, Spada C et al.: Levofloxacin-based triple therapy in first-line treatment for *Helicobacter pylori* eradication. Am J Gastroenterol, 2006, 101, 1985–1990.
33. Pieniazek P, Karczewska E, Duda A, Tracz W, Pasowicz M, Konturek SJ: Association of *Helicobacter pylori* infection with coronary heart disease. J Physiol Pharmacol, 1999, 50, 743–751.
34. Stassen FR, Vainas T, Bruggeman CA: Infection and atherosclerosis. An alternative view on an outdated hypothesis. Pharmacol Rep, 2008, 60, 85–92.
35. Tepes B: Can gastric cancer be prevented? J Physiol Pharmacol, 2009, 60, 71–77.
36. Wotherspoon AC: Gastric MALT lymphoma and *Helicobacter pylori.* Yale J Biol Med, 1996, 69, 61–68.
37. Ziemniak W: Efficacy of *Helicobacter pylori* eradication taking into account its resistance to antibiotics. J Physiol Pharmacol, 2006, 57, 123–141.
38. Zullo A, Perna F, Hassan C, Ricci C, Saracino I, Morini S, Vaira D: Primary antibiotic resistance in *Helicobacter pylori* strains isolated in northern and central Italy. Aliment Pharmacol Ther, 2007, 25, 1429–1434.
39. Zullo A, Vaira D, Vakil N, Hassan C, Gatta L, Ricci C, De Francesco V et al.: High eradication rates of *Helicobacter pylori* with a new sequential treatment. Aliment Pharmacol Ther, 2003, 17, 719–726.

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