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HELICOBACTER PYLORI ANTIBACTERIAL RESISTANCE PATTERNS IN LATVIA: RESULTS FROM THE GISTAR PILOT STUDY

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Abstract – *Objective:* The choice of the *Helicobacter pylori* (*H. pylori*) eradication regimen should depend on the antibiotic resistance of this major gastric pathogen. In Latvia, resistance patterns of *H. pylori* strains have not been described in the general population. The aim of the study is to evaluate the primary susceptibility of *H. pylori* to antibiotics used in recommended eradication regimens for the middle-aged population in Latvia.

Materials and Methods: *H. pylori* antibiotic resistance was assessed in asymptomatic individuals aged 40-64 years, recruited as part of the GISTAR pilot study. *H. pylori* culture was recovered from initially frozen biopsies taken during the endoscopic examinations. Antimicrobial susceptibility to metronidazole, clarithromycin, amoxicillin, tetracycline, rifampicin, and levofloxacin was successfully determined in 89 isolates using the Epsilomer test (E-test, BioMerieux, France), and interpreted according to EUCAST clinical breakpoint standard.

Results: Among the 89 tested isolates, 49 isolates (55%) were resistant to at least one of the tested antibiotics. Resistance to metronidazole was detected in 31 cases (34.8%; 95% CI: 25.2-45.7), followed by rifampicin in nine cases (10.1%; 95% CI: 5.0-18.8%), clarithromycin in five (5.6%; 95% CI: 2.1-13.2) and to levofloxacin in four cases (4.5%; 95% CI: 1.5-11.8). None of the cultures displayed resistance to tetracycline or amoxicillin. Eight resistance patterns were detected: 34 participants had resistance to one of the tested antibiotics; six participants had double resistance, and one participant had resistance to metronidazole, clarithromycin, and levofloxacin.

Conclusions: *H. pylori's* primary resistance to clarithromycin is still low; therefore, the use of standard clarithromycin-based triple therapies may be justified as the first-line therapy for *H. pylori* eradication in the middle-aged population in Latvia.

Keywords: *H. pylori*, Antibiotic resistance, Eradication, Antrum biopsies.

INTRODUCTION

With the formal recognition of chronic infection with *H. pylori* as an infectious disease, its treatment is recommended for *H. pylori*-positive patients, including asymptomatic patients[1]. *H. pylori* treatment suppresses the infection in many cases, even if eradication fails, which commonly happens due to antibiotic resistance. Clearance has been defined as the absence of the bacteria at the end of treatment, and eradication is the absence of bacteria after a period of 4-6 weeks following treatment[2]. A screen-and-treat approach for *H. pylori* infection has been recommended, especially in areas with high gastric cancer burden[1-4]. The results achieved by eradication therapy are population-specific and not transferable. Subsequently, it is important that local *H. pylori* resistance pattern is determined and taken into consideration when selecting an appropriate eradication regimen[1].

Globally, *H. pylori* resistance to antibiotics has reached alarming levels[5]. Based on a systematic review and meta-analyses of 178 studies comprising 66,142 isolates from 65 countries[5], primary and secondary resistance rates to clarithromycin, metronidazole, and levofloxacin were ≥15% (i.e., suggested threshold for choosing alternative empiric regimens[1,2]) in all six regions defined by the World Health Organization (WHO). The exceptions were primary clarithromycin resistance in the Americas (10%; 95% CI: 4%-16%) and South-East Asia region (10%; 95% CI: 5%-16%) and primary levofloxacin resistance in the European region (11%; 95% CI: 9%-13%). Between 2006 and 2016, the resistance rates to these antibiotics increased in all six WHO regions[5]. In addition to regional heterogeneity in resistance rates and patterns, cross-country differences exist. Hence, local surveillance networks are recommended for selecting appropriate eradication regimens and tailoring treatment of *H. pylori* infection based on systematic antimicrobial susceptibility testing. Such approach would limit the increase of global antibiotic resistance by avoiding the use of unnecessary antibiotics[1].

Several studies[5,6] have documented geographic differences in *H. pylori* antibiotic resistance rates and patterns. For example, based on a 2018 study of 1,211 *H. pylori* culture-positive patients from 24 centers in 18 European countries, resistance rates were significantly higher in Central/Western and Southern than in Northern regions[6]. Specifically, primary clarithromycin rates ranged from 4.8% in Denmark to 36.9% in Italy; primary levofloxacin resistance rates ranged from nil in Denmark and the Netherlands to 29.2% in Italy. Overall, 43.0% of *H. pylori* isolates displayed a fully susceptible phenotype. Resistance to one of the antibiotic classes was observed in *H. pylori* isolates from 37.3% of patients. Dual resistance was observed in 16.6% of patients with the most common combinations of clarithromycin and metronidazole resistance, levofloxacin and metronidazole resistance, or clarithromycin and levofloxacin resistance. This study reported aggregated results for 18 European countries, with the exception of country-specific results for clarithromycin and levofloxacin resistance. Fourty-four patients from Latvia represented 3.6% of the study population. The primary clarithromycin resistance of *H. pylori* was 6.8%, and the primary levofloxacin resistance of *H. pylori* was 4.5% in patients from Latvia[6].

Published peer-reviewed studies on the prevalence of *H. pylori* resistance in Latvia or other Baltic states are scant. In 2010, a study in Lithuania reported primary *H. pylori* resistance rates were 35.6% and 20.7% for metronidazole, 3.3% and 16.8% for clarithromycin, and 6% and 0% for ciprofloxacin in adults and children, respectively[7]. The purpose of this study was to estimate the primary *H. pylori* resistance pattern in Latvian middle-aged participants using more recent data and provide first-line treatment recommendations for further *H. pylori* eradication efforts in the country.

MATERIAL AND METHODS

Study Population

This pilot study was performed within the GISTAR Pilot study, which was completed prior to the main study (*H. pylori* eradication and pepsinogen testing for prevention of gastric cancer mortality – the GISTAR study). Its study design is detailed elsewhere[8,9]. Briefly, apparently healthy individuals aged 40-64 years at the time of recruitment were invited to participate in the GISTAR study. To date, it has been carried out in six medium-sized regional towns in Latvia where study centers were created. Data and biological samples used in this study were obtained from

individuals who agreed to participate in the study by attending the GISTAR centers located in the cities of Ludza and Saldus between April 23, 2015, and May 27, 2016. Individuals were asked to fill out a questionnaire on socioeconomic factors, lifestyle and dietary habits, medical history, and phenotype data. Individuals who self-reported receipt of *H. pylori* eradication therapy in the past or undergoing proton pump inhibitor (PPI) treatment or use of antibacterial medications, or medications, or bismuth-containing drugs one month before endoscopy were excluded from the study, as recommended by the *Maastricht* guidelines[2,10]. Individuals with decreased pepsinogen levels (PgI/PgII ≤2 and PgI≤30 ng/mL) were referred for upper endoscopy with a biopsy work-up according to the updated Sydney system[8].

Only those subjects in whom any of two modified-Giemsa-stained antral biopsies had indicated the presence of *H. pylori* infection remained for evaluation of the primary susceptibility to antibiotics.

Biopsy Collection and Storage

In addition to the biopsies obtained routinely for histology, one antral biopsy from the major curvature was used for the current study for culture. Biopsy samples were immediately transferred to sterile 2 ml vials, which contained a specialized media [25 g/L bovine serum albumin (BSA), 74 g/L saccharose, 3.7 mM KH_2PO_4, 6.9 mM K_2HPO_4, 3.6 mM sodium glutamate and water] for improving *H. pylori* survivability and stored in -80^0 C until cultured for *H. pylori*.

Bacterial Culture and Antibiotic Susceptibility Testing

To obtain primary *H. pylori* cultures, a frozen antrum biopsy sample was thawed, mechanically homogenized in a homogenizer pestle (Biomasher™, Japan), and 100 *µ*l of the homogenate was spread onto an *H. pylori* selective media (BioMerieux, France). The plates were cultivated at 37°C for up to 10 days in microaerobic conditions using gas generators (Genbox Microaer Gas Packs, BioMerieux, France) and with a visual inspection every two days. *H. pylori* colonies were identified *via* their morphology – small (0.5-2 mm), round, translucent, and non-hemolytic activity. Definitive identification of *H. pylori* was made with gram staining (gram-negative, helix-shaped, curved rod) and biochemical tests – positive oxidase, catalase, and urease reactions. Colonies from a positively identified culture were collected to create heavy *H. pylori* suspensions in cryogenic storage tubes with 20% glycerol (Viabank, UK) and stored at -80°C. The remaining colonies from the primary cultivation plate were used for antibiotic susceptibility testing.

The Epsilomer test (E-test) strips (BioMerioux, France) were used to determine *H. pylori* susceptibility to metronidazole, clarithromycin, amoxicillin, tetracycline, rifampicin, or levofloxacin. A suspension conforming to McFarland opacity standard was prepared using the remaining colonies from the primary culture in a brain-heart infusion broth (Becton-Dickinson, USA). A freshly prepared Mueller Hinton agar, supplemented with 10% sheep blood, was inoculated with the prepared bacterial suspension and spread evenly across the agar surface. E-test strips were added, and plates were incubated at 37°C in a microaerobic atmosphere for 48 hours. Minimum inhibitory concentration (MIC) (ng/ml) against metronidazole, rifampicin, clarithromycin, and levofloxacin was determined. The results were interpreted according to the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints: >8 ng/ml for metronidazole, >0.25-0.5 ng/ml for clarithromycin, >0.125 ng/ml for amoxicillin, >1 ng/ml for tetracycline, rifampicin and levofloxacin[11,12].

Ethics

All study participants provided written informed consent prior to study enrolment. The GISTAR study was approved by the Central Medical Ethics Committee in Latvia (reg. No. 01-29.1/11) and the ethics committees at the International Agency for Research on Cancer of the World Health Organization (IARC/WHO) (reg. No. IEC 12-36) and Riga East University Hospital (reg. No. 14-A/13).

Statistical Analysis

E-test results were interpreted according to the EUCAST clinical breakpoint standard (https://www.eucast.org/, version 6.0), and frequency counts, percentages, and corresponding 95% confidence intervals (CIs) were calculated using WHONET, a free software developed by the WHO Collaboration Centre for Surveillance of Antimicrobial Resistance for laboratory-based surveillance of infectious diseases and antimicrobial resistance (https://www.who.int/, version 5.6).

RESULTS

Patient Samples

Biopsy samples from 133 participants were analyzed, and antimicrobial susceptibility to metronidazole, clarithromycin, amoxicillin, tetracycline, rifampicin, and levofloxacin was successfully determined in isolates from 89 participants (50.6% men). The mean age of study participants was 51 years (±6.7 SD), ranging from 40 to 64 years. Sixty-two participants (69.7%) resided in the city of Saldus and 27 (30.3%) in Ludza.

H. pylori resistance Patterns

Among 89 participants, 49 (55.1%) were resistant to at least one of the 6 antibiotics (Table 1). The most common resistance was to metronidazole, detected in 31 cases (34.8%; 95% CI: 25.2%-45.7%), followed by 9 isolates (10.1%; 95% CI: 5.0%-18.8%) that were rifampicin-resistant, 5 isolates (5.6%; 95% CI: 2.1%-13.2%) with resistance to clarithromycin and four isolates (4.5%; 95% CI:1.5%-11.8%) to levofloxacin. None of the isolates were resistant to tetracycline or amoxicillin. The rate of metronidazole resistance was 40.0% (18/45) in men and 29.5% (13/44) in women. More details are provided in Figure 1. In Ludza and Saldus, metronidazole resistance rates were 51.9% and 27.4%, respectively (Figure 2).

The following resistance patterns were observed among isolates: 34 were resistant only to one of the tested antibiotics, from which 24 isolates (27%) were metronidazole-resistant; two (2%) were clarithromycin-resistant; two (2%) were levofloxacin-resistant, and six isolates

TABLE 1. *H. PYLORI* RESISTANCE TO THE ANTIBIOTICS TESTED IN THE STUDY (N=89).

	Antibiotic	Number of resistant isolates	Percentage of resistant isolates (%)	95% CI
Saldus (N=62)	Metronidazole	17	27.4	17.2-40.4
	Rifampicin	8	12.9	6.1-24.4
	Clarithromycin	4	6.5	2.1-16.6
	Levofloxacin	2	3.2	0.6-12.1
	Amoxicillin	0	0.0	0.0-7.3
	Tetracycline	0	0.0	0.0-7.3
Ludza (N=27)	Metronidazole	14	51.9	32.4-70.9
	Rifampicin	1	3.7	0.2-20.9
	Clarithromycin	1	3.7	0.2-20.9
	Levofloxacin	2	7.4	1.3-25.7
	Amoxicillin	0	0.0	0.0-15.5
	Tetracycline	0	0.0	0.0-15.5
Total (N=89)	Metronidazole	31	34.8	25.2-45.7
	Rifampicin	9	10.1	5.0-18.8
	Clarithromycin	5	5.6	2.1-13.2
	Levofloxacin	4	4.5	1.5-11.8
	Amoxicillin	0	0.0	0.0-5.2
	Tetracycline	0	0.0	0.0-5.2

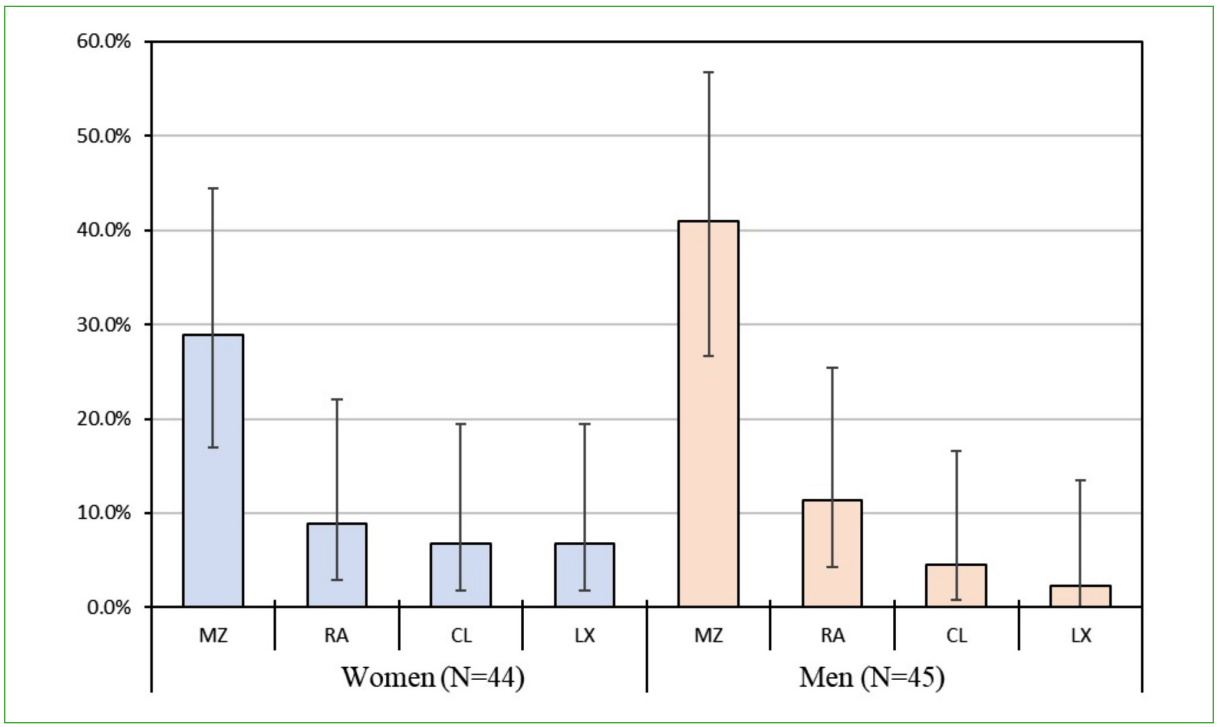

Figure 1. *H. pylori* resistance in study participants by sex. MZ – metronidazole, CL – clarithromycin, AX – amoxicillin, TC – tetracycline, RA – rifampicin, LX- levofloxacin. Error bars represent 95% confidence intervals.

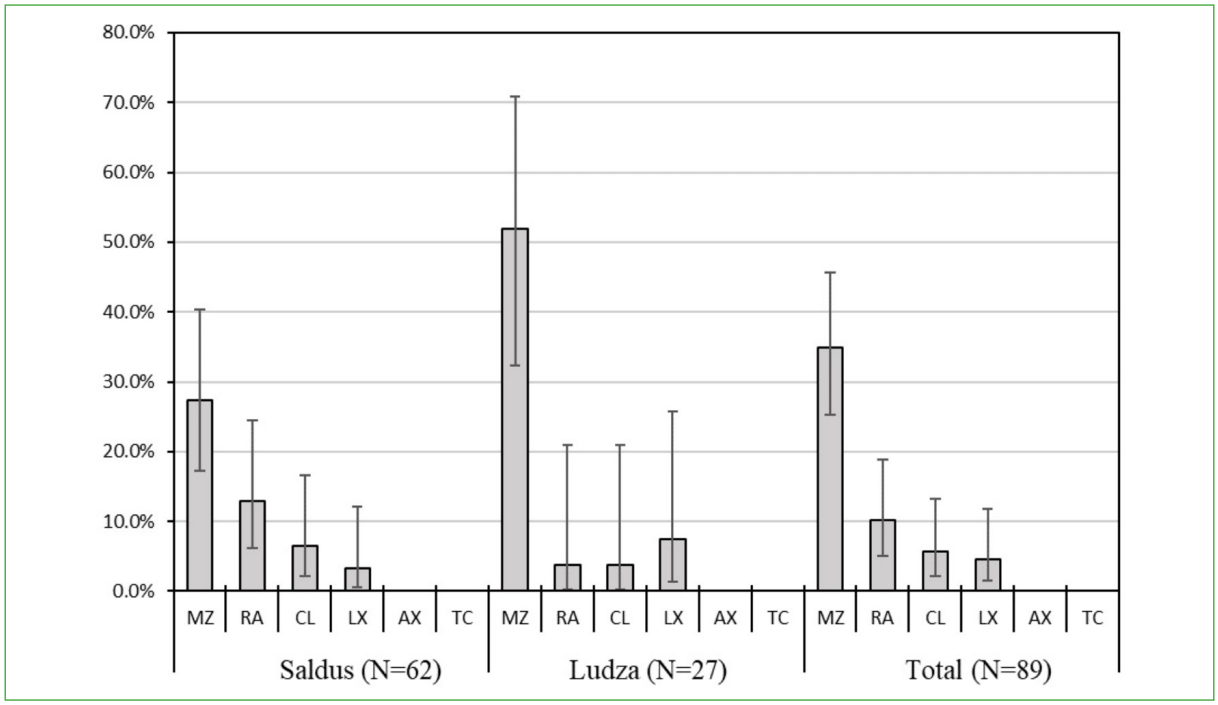

Figure 2. *H. pylori* resistance to the antibiotics tested in the study among participants by city of residence and overall. MZ – metronidazole, CL – clarithromycin, AX – amoxicillin, TC – tetracycline, RA – rifampicin, LX- levofloxacin.

(6%) were rifampicin-resistant. Six isolates had double resistance: two (2%) were resistant to metronidazole and clarithromycin; one (1%) was resistant to metronidazole and levofloxacin, and three isolates (3%) were resistant to metronidazole and rifampicin. One isolate (1%) had resistance to metronidazole, clarithromycin and levofloxacin (Table 2).

TABLE 2. OBSERVED *H. PYLORI* RESISTANCE PATTERNS.

Resistance profile	Number of isolates (N=49)	% of isolates
Metronidazole	24	27.0
Metronidazole and rifampicin	3	3.4
Metronidazole and clarithromycin	2	2.2
Metronidazole, clarithromycin and levofloxacin	1	1.1
Metronidazole and levofloxacin	1	1,1
Rifampicin	6	6.7
Clarithromycin	2	2.2
Levofloxacin	2	2.2

MIC Distribution

The MIC distribution for the tested antibiotics in both centers (or cities) is shown in Table 3. Among the metronidazole-resistant isolates, 27 out of 31 (88%) showed a high-level resistance (≥256 μg/ml). Clarithromycin had a high resistance pattern (≥ 256 μg/ml) in three out of five cultures. The average MIC value for the remaining two clarithromycin-resistant isolates was 1.125 μg/ml (±0.5 SD). High rifampicin resistance (≥ 32) was observed in two isolates, and an average MIC value of 5 μg/ml (±4,7 SD) was estimated in 7 isolates. For levofloxacin-resistant isolates, the average MIC value was 3.38 μg/ml (±31.2 SD), with two out of four presenting a high-level resistance.

DISCUSSION

Using data from the pilot study[9], conducted within the GISTAR study[8], we have examined *H. pylori* antibacterial resistance rates among middle-aged Latvia adults. While attempts were made to assess *H. pylori* sensitivity to antibiotics in the country in the past[13], this study is the first one to comprehensively document the situation in the general population in Latvia.

TABLE 3. DISTRIBUTION OF MINIMUM INHIBITORY CONCENTRATIONS OF METRONIDAZOLE, RIFAMPICIN, CLARITHROMYCIN AND LEVOFLOXACIN FOR *H. PYLORI* BY PARTICIPANTS' CITY OF RESIDENCE.

Antibiotic	MIC† (ng/ml)	Saldus (n=62)	Ludza (n=27)	Total (n=89)	Min-Max (ng/ml)	Average (ng/ml)
Metronidazole	≤ 8 > 12 ≥ 256	45 3 14	13 1 13	58 4 27	0.016-6 12-48	0.318 31
Rifampicin	≤ 1 > 1 ≥ 32	54 6 2	26 1 —	80 7 2	0.047-1 1.5-12	0.36 5
Clarithromycin	≤ 0.25 > 0.25-≤ 0.5 ≥ 256	58 2 2	26 — 1	84 2 3	0.016-0.047 0.75-1.5	0.017 1.125
Levofloxacin	≤ 1 > 1 ≥ 32	60 1 1	25 1 1	85 2 2	0.003-0.094 2-8	0.03 5

†MIC – Minimum Inhibitory Concentration.

A prospective study[14] conducted from April 2008 to June 2009 addressed the primary *H. pylori* antibiotic resistance in 18 European countries, excluding Latvia. Of 2,204 adult patients included in the study overall, *H. pylori* resistance to clarithromycin was 17.5%, which is considered high, requiring the use of a bismuth-based quadruple therapy as the first option. If bismuth is not available, a non-bismuth quadruple therapy can be used, preferably as a sequential therapy[1,15]. However, in Finland and Lithuania, neighboring countries of Latvia, lower resistance rates to clarithromycin were identified[14]. Similar to the 2018 European study[6], our study has revealed *H. pylori* resistance rates to clarithromycin of 5.6%, which is considered low, justifying continued use of standard triple therapy as a first-line treatment[1,15].

When we collected data for this study, macrolide group antibiotics were the third most commonly prescribed group of antibacterial drugs, preceded by penicillin and tetracycline group antibiotics in Latvia[16]. Therefore, a relatively lower consumption of macrolides (1.81 DDD/1000 inhabitants/day in 2016) could explain the low resistance to clarithromycin in Latvian patients[16]. Clarithromycin MIC values also showed a clear bimodal pattern, with 75% of cultures having a high-level resistance, which conforms to findings in previous studies[17,18].

In terms of metronidazole resistance, we have found it to be the most common among tested antibiotics in our study participants (34.8%). In the aforementioned prospective study of 18 European countries, including Latvia, 38.9% of patients were resistant to metronidazole, and there has been a significant increase since 2008[14]. Lack of adherence to drug prescription regulations coupled with the low cost of metronidazole could be the cause of high metronidazole consumption among adults in Latvia.

The relatively high observed rifampicin resistance (10.1%) could be due to the susceptibility testing methodology and respondent's medical history, for example, past tuberculosis treatments. We have observed some of the isolates had the tendency to develop rifampicin resistance simply when cultivated for an extended period 48-72h (results not shown). A similar phenomenon was also observed by Glocker et al[19] when, after a single rifampicin selection step, they obtained spontaneous rifampicin-resistant mutants from a rifampicin-susceptible strain 26695. Based on this observation, it is possible for the rifampicin resistance rates to be overestimated.

Even though levofloxacin has not been widely used in Latvia, we observed a primary *H. pylori* resistance to this antibiotic of 4.5%. Over the years, the use of quinolone group antibiotics in Latvia has been higher than in neighboring European countries and Norway, e.g., in 2017, 1.03 DDD per 1000 inhabitants and per day in Latvia, compared to 0.79, 0.87, and 0.35 DDD/1000 inhabitants/day in Estonia, Lithuania, and Norway, respectively, in 2017[16]. In Portugal, where the use of quinolone group antibiotics was only recently reduced to 1.25 DDD/1000 inhabitants/day (previous usage spanned from 2.2 to 1.9 between 2013 and 2016), the primary levofloxacin resistance rate was 15.4% in 2014[20]. A similar trend can be observed in Italy, where quinolone consumption in 2016 was even higher at 3.2 DDD/1000 inhabitants/day, whose effects can also be seen in high resistance to levofloxacin, with the rate being approximately 30%[21]. Based on a most recent study[6] of 1,211 adult patients from 24 centers in 18 European countries, the primary levofloxacin resistance of H. pylori was 4.5% in patients from Latvia. The average rate of resistance to levofloxacin was 15.8%, ranging from 29.2% in Italy to nil in the Netherlands and Denmark[6].

The prevalence of *H. pylori* resistance to both clarithromycin and metronidazole (dual resistance) is also an important consideration. Concomitant therapy is ineffective against dual-resistant strains. A recent review found an *H. pylori* infection treatment success rate of only 79%, suggesting that this combination should not be used if the prevalence of dual resistance is >15%. Bismuth-containing quadruple therapy was considered the first-line treatment for areas of high dual resistance in the latest European consensus report[1]. Other regimens potentially useful in this situation would be high-dose PPI-amoxicillin dual therapy or rifabutin triple therapy, as they avoid the issue of clarithromycin and metronidazole resistance altogether. Resistance to rifabutin or amoxicillin was nil in our study.

Limitations and Strengths

Although we had high success in recovering *H. pylori* from frozen biopsies, the relatively small size of the study group needs to be mentioned as a limitation of our study. An important strength of this study includes the use of isolates from healthy participants from the general population rather than those from symptomatic patients.

CONCLUSIONS

In conclusion, in the studied Latvian middle-aged population, the primary *H. pylori* resistance to clarithromycin has remained low. Therefore, the use of standard clarithromycin-based eradication regimens may still be used as first-line treatment, at the very least, in the regions and age groups investigated within this study. Antimicrobial susceptibility testing employing next-generation sequencing methods to increase throughput would be advised for better estimation of resistance patterns.

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Conflict of Interest

The authors declare that they have no conflict of interest. Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article, and they do not necessarily represent the decisions, policies, or views of the International Agency for Research on Cancer /World Health Organization.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Statement

The study was approved by the Central Medical Ethics Committee in Latvia (reg. No. 01-29.1/11) and the ethics committees at the International Agency for Research on Cancer (IARC) (reg. No. IEC 12-36) and Riga East University Hospital (reg. No. 14-A/13).

Informed Consent

The written informed consent was obtained from all the participants in the study.

Authors' Contributions

Dace Rudzīte performed laboratory work, analysis, and interpretation of results.
Reinis Vangravs conducted data collection, analysis, interpretation of results, and manuscript preparation.
Ģirts Šķenders performed interpretation of results and manuscript editing.
Inese Poļaka conducted data analysis.
Dārta Pūpola performed interpretation of results.
Ilva Daugule performed analysis and manuscript preparation.
Aigars Vanags conducted endoscopies and biopsies, reviewed manuscript, and provided clinical support.
Juris Atstupens conducted endoscopies and biopsies, reviewed manuscript, and provided clinical support.
Yelena Tarasenko prepared and revised the manuscript.
Jin Young Park designed the project and prepared the manuscript.
All authors contributed to the manuscript preparation and approved the final version of the manuscript.

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REFERENCES

1. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, Gasbarrini A, Hunt RH, Leja M, O'Morain C, Rugge M, Suerbaum S, Tilg H, Sugano K, El-Omar EM. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut 2022; 71: 1724-1762.
2. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. Gut 2017; 66: 06-30.
3. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, Haruma K, Asaka M, Uemura N, Malfertheiner P. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut 2015; 64: 1353-1367.
4. Li H, Yang T, Tang H, Tang X, Shen Y, Benghezal M, Tay A, Marshall B. *Helicobacter pylori* infection is an infectious disease and the empiric therapy paradigm should be changed. Precis Clin Med 2019; 2: 77-80.
5. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in *Helicobacter pylori*: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology 2018; 155: 1372-1382.e17.
6. Megraud F, Bruyndonckx R, Coenen S, Wittkop L, Huang TD, Hoebeke M, Bénéjat L, Lehours P, Goossens H, Glupczynski Y. *Helicobacter pylori* resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. Gut 2021; 70: 1815-1822.
7. Rudzite D, Sudraba A, Daugule I, Funka, K. Tolmanis, I. Vanags, A. Engstrand, L. Janciauskas, D. Jonaitis, V. Kupcinskas, L. Ivanauskas, A. Leja M. Antimicrobial susceptibility pattern of *H. Pylori* strains isolated from adult dyspeptic patients in Latvia. Helicobacter: XXIII International Workshop on Helicobacter and Related Bacteria in Chronic Digestive Inflammation and Gastric Cancer : Rotterdam, 2010: Abstracts / European Helicobacter Study Group. Cambridge, MA: Wiley-Blackwell Science 2010; 15: 392, P8.15.
8. Leja M, Park JY, Murillo R, Liepniece-Karele I, Isajevs S, Kikuste I, Rudzite D, Krike P, Parshutin S, Polaka I, Kirsners A, Santare D, Folkmanis V, Daugule I, Plummer M, Herrero R. Multicentric randomised study of *Helicobacter pylori* eradication and pepsinogen testing for prevention of gastric cancer mortality: the GISTAR study. BMJ Open 2017; 7: e016999.
9. Park JY, Polaka I, Parshutin S, Kikuste I, Isajevs S, Santare D, Rudzite D, Vanags A, Liepniece-Karele I, Kirsners A, Atstupens J. Trial profile: pilot study of the multicentre randomised trial of *H. Pylori* eradication and pepsinogen testing for prevention of gastric cancer mortality (the GISTAR Pilot study). Microb Health Dis 2019; 1: e165.
10. Skrebinska S, Megraud F, Daugule I, Santare D, Isajevs S, Liepniece-Karele I, Bogdanova I, Rudzite D, Vangravs R, Kikuste I, Vanags A, Tolmanis I, Savcenko S, Alix C, Herrero R, Park JY, Leja M. Who Could Be Blamed in the Case of Discrepant Histology and Serology Results for *Helicobacter pylori* Detection? Diagnostics 2022; 12: 133.
11. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI M100. Clinical and Laboratory Standards Institute, 2019. Available at: https://www.clsi.org/
12. European Committee on Antimicrobial Susceptibility Testing. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters v10.0. EUCAST Clinical Breakpoint Tables, 2020. Available at: https://eucast.org/clinical_breakpoints/
13. Rudzite D, Leja K, Kikuste I, Aiga R, Vangravs R, Santare D, Pūpola D, Skenders G, Leja M. Antimicrobial Susceptibility in *Helicobacter pylori* Isolated from Gastric Biopsies in Adult Population in Latvia. XXXth International Workshop on Helicobacter & Microbiota in Inflammation and Cancer, 2017.
14. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut 2013; 62: 34-42.
15. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of *Helicobacter pylori* infection--the Maastricht IV/ Florence Consensus Report. Gut 2012; 61: 646-664.
16. European Centre for Disease Prevention and Control. Antimicrobial Consumption – Annual Epidemiological Report for 2022. Table D6. Trends in consumption of quinolones (ATC group J01M) in the community, EU/EEA countries, 2013-2022. Expressed as DDD per 1,000 inhabitants per day. 17 November, 2023. Available at: https://www.ecdc.europa.eu/en/publications-data/downloadable-tables-antimicrobial-consumption-annual-epidemiological-report-2022
17. Serrano CA, Leon MA, Palma C, Vera M, Hernandez C, Harris PR. *Helicobacter pylori*- Clarithromycin Resistance in Symptomatic Pediatric Patients in a High Prevalence Country. J Pediatr Gastroenterol Nutr 2017; 64: e56-e60.
18. Ho SL, Tan EL, Sam CK, Goh KL. Clarithromycin resistance and point mutations in the 23S rRNA gene in *Helicobacter pylori* isolates from Malaysia. J Dig Dis 2010; 11: 101-105.
19. Glocker E, Bogdan C, Kist M. Characterization of rifampicin-resistant clinical *Helicobacter pylori* isolates from Germany. J Antimicrob Chemother 2007; 59: 874-879.
20. Lopo I, Libânio D, Pita I, Dinis-Ribeiro M, Pimentel-Nunes P. *Helicobacter pylori* antibiotic resistance in Portugal: Systematic review and meta-analysis. Helicobacter 2018; 23: e12493.
21. Fiorini G, Zullo A, Saracino IM, Pavoni M, Vaira D. Antibiotic resistance pattern of *Helicobacter pylori* strains isolated in Italy during 2010-2016. Scand J Gastroenterol 2018; 53: 661-664.