ORIGINAL ARTICLE

Helicobacter pylori resistance to clarithromycin and quinolones in patients with dyspepsia in Tuzla Canton, Bosnia and Herzegovina

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ABSTRACT

Aim To evaluate *Helicobacter pylori* (*H. pylori*) resistance to clarithromycin and quinolones in patients with dyspepsia in Tuzla Canton, Bosnia and Herzegovina, a region with no data on clarithromycin or quinolones resistance.

Methods A prospective cross-sectional study was conducted at the Department of Gastroenterology and Hepatology at University Clinical Centre Tuzla between January 2021 and June 2022. The study included 99 patients who underwent esophagogastroduodenoscopy (EGDS) due to dyspepsia. In all patients biopsies were taken for rapid urease test (RUT) and histology findings, concomitantly with blood samples for IgG serology. All RUT positive patient samples were tested for clarithromycin and quinolones susceptibility with GenoType HelicoDr, a PCR method which detects point mutations in 23S rRNA and mutations in the gyrA gene.

Results Out of 99 dyspeptic patients, 67 (67.7%) were serologically positive to *H. pylori*, 46 (46.4.%) were RUT positive, and 19 (19.2 %) had a positive histology finding. Antibiotic (AB) resistance was tested in the total of 46/99 (46.4%) patients. Resistance to clarithromycin was detected in 28.26% (13/46), quinolones resistance in 36.96% (17/46) , and resistance to both AB was detected in 8.69% (4/46) tested biopsies.

Conclusions Due to high clarithromycin and quinolones resistance rates, we recommend the use of bismuth quadruple or non-bismuth concomitant quadruple therapy for *H. pylori* eradication in Tuzla Canton, Bosnia and Herzegovina.

Key words: 23S rRNA, gyrA, HelicoDR, mutations, RUT, susceptibility

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INTRODUCTION

Currently, at least half of the world's population is infected with *Helicobacter pylori* (*H. pylori*) (1), but the infection is more common in undeveloped and developing countries such as Bosnia and Herzegovina (B&H). In the new published Maastricht VI-Florence consensus report *H. pylori* infection is formally recognised as an infectious disease and is now included in the International Classification of Diseases 11th revision (2) meaning that all infected patients (including patients without symptoms) should receive a treatment. The bacterium is mainly isolated from biopsy material from the stomach, but it can also be detected in saliva, gastric emesis and faeces. More than 80% of infected persons do not have any symptoms (3). *H. pylori* can cause gastritis, gastric cancer and lymphoma. According to the International Agency for Research on Cancer (1994), the World Health Organization (WHO) classified *H. pylori* as Group 1 carcinogen in humans (4). Gastric adenocarcinoma is the fifth most frequent and third malignant neoplasm in the world (5).

For proper diagnosis of the infection, invasive (Rapid Urease Test, histology, culture and polymerase chain reaction) and non-invasive tests (serology, Urea Breath Test, Stool Antigen Test) could be performed (2). The last two decades have been marked with increased resistance rates of *H. pylori* to macrolides, such as clarithromycin, but also to quinolones, metronidazole and amoxicillin, all common antibiotics used for *H. pylori* eradication (5). Early and proper diagnosis of *H. pylori* and knowing the regional antibiotic resistance are important for proper antibiotic therapy. It is mandatory for each region in the World to evaluate the clarithromycin resistance in order to choose the proper therapy for *H. pylori* eradication (2).

Clarithromycin is a bacteriostatic antibiotic that belongs to the macrolide family. It is one of the most potent antibiotics against *H. pylori*, but is limited by the increasing rate of resistance to it (6). The main mechanism of action of clarithromycin in *H. pylori* therapy is to prevent protein translation. Clarithromycin resistance due to point mutations in the 23SrRNA component of ribosomes is continuously increasing, and is supposed to be the leading cause of eradication regimen failures. However, two major mutations A2146G and A2147G are listed as main causes of antibiotic resistance in clinical isolates (7).

Quinolones are members of a large group of broad spectrum bactericidal antibiotics hat share a bicyclic core structure related to the substance 4-quinolone. Levofloxacin and moxifloxacin are often used as a second line therapy for *H. pylori* eradication, sometimes also as first line. Ciprofloxacin is one of the most used antibiotics worldwide. The mutations on the gyrA gene 87 (mostly mutations on the position N87K) and gyrA 91 (position D91N) are responsible for the quinolones resistance (8). Empirical treatments include the combination of antibiotics and proton pump inhibitor (PPI) or bismuth. Clarithromycin triple therapy with amoxicillin, clarithromycin and PPI is the most frequently used first-line treatment for *H. pylori* eradication in regions with clarithromycin resistance lower than 15%. Until now there have been no data for clarithromycin resistance or any other antibiotic resistance used for *H. pylori* treatment in B&H. Furthermore, in B&H there is no routine real time (RT) PCR testing; the main diagnostic tests in our hospitals are serology, RUT and histology.

The aim of this study was to assess prevalence of *H. pylori* resistance to quinolones and clarithromycin, as well as to compare these three diagnostic tests in patients referred for upper GI endoscopy due to dyspeptic symptoms.

PATIENTS AND METHODS

Patients and study design

A prospective cross-sectional study was conducted at the Department of Gastroenterology and Hepatology at the University Clinical Centre Tuzla between January 2021 and June 2022. This study included 99 patients who underwent esophagogastroduodenoscopy (EGDS) due to dyspepsia selected from a total of 360 dyspeptic patients.

Symptom duration was defined as at least one day of early satiety and post-prandial fullness, or three days of epigastric pain/burning per week during the last three months with symptoms onset at least six months previously. Exclusion criteria were patients with malignancies, patients who received *H. pylori* eradication treatment previously (clarithromycin triple therapy or levo-

floxacin based therapy), as well as the treatment with proton pump inhibitors and/or antibiotics or bismuth salts in the previous two and four weeks, respectively. After the exclusion, 99 patients left who fulfilled the criteria.

The study protocol was approved by the Ethics Committee of University Clinical Centre Tuzla. Signed consent forms were obtained from all patients.

Methods

Screening for *H. pylori* infection was performed in all patients by Rapid Urease Test (Pronto Dry), which detects the urease enzyme of *H. pylori* if there are at least 105 bacteria per biopsy sample. Blood samples were taken from all patients in the Microbiology Department for *H. pylori* ELISA IgG testing (Enzygnost Anti Helicobacter Pylori II IgG, Siemens, Munich, Germany). Esophagogastroduodenoscopy was performed by a gastroenterologist for every patient on Olympus Exera III (Olympus, Tokyo, Japan). Five biopsies were taken following the Sidney protocol (2) (2 biopsies from antrum, 2 from the corpus, 1 from the incisura angularis) and one biopsy was taken for RUT (from the antrum). All biopsies were sent to the Pathology Department for histology findings. The biopsy specimens were fixed in 10% formalin and Giemsa staining was used to confirm the presence or absence of *H. pylori*. All positive RUT biopsies were sent to the Microbiology Department as well, and stored at -20°C in a freezer until the extraction of DNA.

H. pylori antibiotic resistance to clarithromycin and quinolones was tested with PCR Detection Kit (GenoType HelicoDR, Hain Lifescience, Nehren, Germany), which included DNA extraction from the biopsy samples using the QIAmp DNA Mini Kit (Qiagen, Benelux, The Netherlands), amplification with biotinylated primers and reverse hybridisation performed on strips prepared from the manufacturer using DNA strip technology (9). Interpretation of susceptibility to clarithromycin and levofloxacin was defined as hybridization of the wild-type (WT) probe with the absence of mutant probes. The absence of hybridization of any WT or mutant gene was interpreted as resistance to these drugs. The simultaneous presence of WT and mutant bands in the same strip was considered a hetero resistance pattern (9).

Statistical analysis

All variables were tested for normal distribution by using Kolmogorov-Smirnoff test. Descriptive data were presented as mean, standard deviations (SD) and a range or percentages. Demographic and clinical data of patients and frequencies of antibiotic resistance were analysed, as well as cross tabulation of data on specific antibiotic resistance. Statistical significance was assumed at $p<0.05$.

RESULTS

There were 99 evaluated patients with median age of 58 (IQR – interquartile range 49-66) years, and age range of 17-76 years. There were 56 (56.6%) females and 43 (43.4%) males (ratio of 1.30:1). Ninety (90.9%) patients had histological confirmation of chronic gastritis. The endoscopic findings were hyperaemic gastropathy in 68 (68.7%) patients, biliary gastropathy in seven (7.1%) and ulcer disease in five (5.0%) patients (Table 1).

Table 1. Demographic and clinical data of 99 patients

Demographic/Clinical data	Value
Mean age (interquartile range) (years)	58 (46-66)
Gender (female/male) (No)	56/43
Ratio (female/male)	1.3:1
Endoscopic findings (No, %)	
Chronic gastritis (total)	90 (90.9)
Hyperaemic gastropathy	68 (68.7)
Biliary gastropathy	7 (7.1)
Bulbar and gastric ulceration	5 (5)

Of 99 tested patients, 67 (67.7%) were serologically positive to *H. pylori*, 46 (46.6%) had a positive RUT, and in 19 (19.2%) patients *H. pylori* was confirmed by histology (Table 2).

Table 2. Frequency of *H. pylori* positivity according to a type of testing

Test results	No (%) of strains		
	Serology IgG	Rapid urease test (RUT)	Histology
Positive	67 (67.7)	46 (46.4)	19 (19.2)
Negative	32 (32.3)	53 (53.6)	80 (80.8)

Antibiotic (AB) resistance was determined by PCR in 46 (out of 99; 46.4%) patients. A total of four (out of 46; 8.69%) *H. pylori* strains showed resistance to both quinolones and clarithromycin. Clarithromycin resistance was noticed in 13 (28.26%), and quinolone resistance in 17 (36.96%) *H. pylori* strains (Table 3).

Table 3. Antibiotic resistance of 46 *H. pylori* strains determined by PCR

Clarithromycin/ Quinolones	No (%) of strains		
	Sensitive	Resistant	Total
Clarithromycin	33 (71.74)	13 (28.26)	46 (100)
Quinolones	29 (63.04)	17 (36.96)	46 (100)
Dual resistance		4 (8.69)	
Total		30 (62.21)	

The most common mutations detected in clarithromycin resistant samples were A2147G, in 84.6% (11/13). Majority of gyrA mutations were observed at codon 91, 52.9% (9/17). The gyr87MUT (N87K) band was detected in 47.1% (8/17) of quinolones resistant samples. A double mutation in codon 91 was observed in 23.5% (4/17) samples (Table 4).

DISCUSSION

In this study 67.7% of dyspeptic patients were serologically positive to *H. pylori*, 46.4.% of all patients were RUT positive and only 19.2 % had a positive histology finding. The infection did not depend on age or gender. In comparison of serology against RUT and histology, it is obvious that serology is useful in excluding the infection due to high sensitivity rates (2). According to our results, specificity of serology is modest, and should not be used in terms of diagnosing the *H. pylori* positivity.

Histology is usually considered to be the gold standard in the direct detection *of H. pylori* infection (2). However, several factors influence the diagnostic accuracy of histology, such as site, size and number of biopsies, staining methods, the use of proton pump inhibitors, antibiotics and experience of the examining pathologist (10). Biopsies from both antrum and corpus are usually recommended in clinical practice and the acquisition of at least two biopsy specimens from antrum and corpus is the most sensible strategy that guarantees the maximum diagnostic yield (2). Staining is the critical part of histological examination and several stains like routine HE staining, Giemsa, Warthine-Starry, Hp silver stain, toluidine blue, acridine orange, McMullen, Genta, Dieterle, and immunohistochemical stain have been used to detect *H. pylori* (10). Giemsa stain had been a preferred method in clinical practice for years, because it is simple, highly sensitive and less expensive, but routine *H. pylori* immunohistochemical staining (IHC) is now the preferred method in most hospitals (10). Giemsa staining is the default method in our hospital for *H. pylori* detection, and the results of only 19.2 % positive histology findings in comparison to 46.4% RUT positive patients could be the repercussion of the staining method and the biopsy site of the taken specimen, although Sydney protocol was fulfilled (2). Due to the focal distribution pattern of the bacterium in the gastric mucosa there is a chance of false negativity even with the most sensitive methods like immunohistochemistry IHC and fluorescence *in situ* hybridization (FISH). As the false detection rate is higher, the bacterial load is lower (10). Additionally, it is important to emphasize that PCR and RUT are able to detect presence of even small quantity of microorganism, thus allowing for better diagnostic yield (2).

Therapeutic management of *H. pylori* is still problematic as many patients remain infected despite several standard drug regimens (1). In a recently published network meta-analysis, the best performing first-line regimen with levofloxacin and amoxicillin reached successful eradication in 88.5% of patients in Western countries (11). Additionally, adding probiotic to eradication protocol enhances outcomes in terms of eradication rate while decreasing the side-effects of the tre-

Table 4. Distribution of genotypes detected according to 23S rRNA and gyrA point mutations

Clarithromycin/ Quinolones	Target gene	No of samples with mutations	Type of mutation	No (%) of positive samples
Clarithromycin	23S rRNA	13	23S-MUT1(A2146G)+23S-MUT2(A2146C)	1 (7.7)
			23S-MUT2(A2146C)	1 (7.7)
			23S-MUT3(A2147G)	11(84.6)
Quinolones	gyrA	17	gyr87MUT (N87K)	8 (47.1)
			gyr91MUT1 (D91N)	4 (23.5)
			gyr91MUT2 (D91G)	3 (17.6)
			gyr91MUT3 (D91Y)	2 (11.8)
Dual resistance	23S rRNA +gyrA	4	23S-MUT1(A2146G)+gyr91MUT1 (D91N)	1
			23S-MUT3(A2147G) +gyr91MUT2 (D91G)	1
			23S-MUT3(A2147G)+gyr91MUT3 (D91Y)	2

atment, most of which pertaining to lower rate of antibiotics associated diarrhoea, abdominal pain and cramping (12).

This is the first study from Bosnia and Herzegovina regarding *H. pylori* antibiotic resistance. The estimated clarithromycin resistance in this study was 28.26%, and this result is expected given that B&H is a developing country and that South-East European countries have a high *H. pylori* seroprevalence (13). A European comparison showed that the resistance is higher in Central/Eastern Europe 9.3%, and it is the highest in Southern Europe, 18% (13).

Recent studies from South-East European countries in the last two years conducted in Greece (14) with the result of 23.2% *H. pylori* clarithromycin resistance, Turkey (15) with 19%, and Bulgaria (16) with 34% confirm the trend of high clarithromycin resistance in this part of Europe. When we compare our results with neighbouring countries, a slightly higher clarithromycin resistance was reported in B&H than in Serbia (24%) (17) and Croatia (21.2%) (18).

Quinolone resistance is also increasing worldwide. The estimated quinolones resistance in this study was 36.96 %. The average European resistance on quinolones is 15.8% (19). When compared to our neighbours, Serbia has only 8.3% quinolone resistance (17), which is quite low compared to the European average (19) and compared to our results. The high quinolone resistance in our country may be explained by the wide use of antibiotics such as levofloxacin and especially ciprofloxacin. The resistance to both antibiotics was 8.69%, which is rather high compared to previous studies (8). However, these results might be due to the more accurate method for detection of clarithromycin and quinolones resistance that was used in this study, namely PCR and uncontrolled use of antibiotics.

Currently, culture and antimicrobial susceptibility tests are not routinely performed in the clinical practice, but susceptibility testing-based tailored therapies will be introduced soon (20).

There are a few limitations in this study that we need to address. First, the sample size which is quite small because of the fact that real time PCR methods and antibiotic resistance on *H. pylori* are not routinely done in our hospital, so we received only a limited number of DNA strips to conduct the quinolones and clarithromycin resistance. Also, it would be better to include more antibiotics such as amoxicillin and metronidazole to test resistance and more *H. pylori* diagnostic tests to compare with (especially Urea Breath Test and Stool Antigen Test).

In conclusion, *H. pylori* resistance in Bosnia and Herzegovina has been unclear until now. Studies on the resistance status of *H. pylori* are needed in order to improve the treatment strategies. The choice of the best *H. pylori* eradication regimen is strongly geographically related, and depends on clarithromycin resistance in a specific region. According to our results, a bismuth quadruple therapy or a non-bismuth concomitant quadruple therapy should be the first-choice therapy in Tuzla Canton. It is also important to know that levofloxacin as the second line therapy in *H. pylori* eradication should be reconsidered in our region.

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TRANSPARENCY DECLARATION

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