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Antimicrobial resistance of *Helicobacter pylori* strains to five antibiotics, including levofloxacin, in Northwestern Turkey

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ABSTRACT

Introduction: Antibiotic resistance is the main factor that affects the efficacy of current therapeutic regimens against *Helicobacter pylori*. This study aimed to determine the rates of resistance to efficacy clarithromycin, amoxicillin**,** tetracycline, levofloxacin and metronidazole among *H. pylori* strains isolated from Turkish patients with dyspepsia. **Methods:** *H. pylori* was cultured from corpus and antrum biopsies that were collected from patients with dyspeptic symptoms, and the antimicrobial susceptibility of *H. pylori* was determined using the E-test (clarithromycin, amoxicillin, tetracycline, metronidazole and levofloxacin) according to the EUCAST breakpoints. Point mutations in the 23S rRNA gene of clarithromycin-resistant strains were investigated using real-time PCR. **Results:** A total of 98 *H. pylori* strains were isolated, all of which were susceptible to amoxicillin and tetracycline. Of these strains, 36.7% (36/98) were resistant to clarithromycin, 35.5% (34/98) were resistant to metronidazole, and 29.5% (29/98) were resistant to levofloxacin. Multiple resistance was detected in 19.3% of the isolates. The A2143G and A2144G point mutations in the 23S rRNA-encoding gene were found in all 36 (100%) of the clarithromycin-resistant strains. Additionally, the levofloxacin MIC values increased to 32 mg/L in our *H. pylori* strains. Finally, among the clarithromycin-resistant strains, 27.2% were resistant to levofloxacin, and 45.4% were resistant to metronidazole. **Conclusions:** We conclude that treatment failure after clarithromycin- or levofloxacin-based triple therapy is not surprising and that metronidazole is not a reliable agent for the eradication of *H. pylori* infection in Turkey.

Keywords: *Helicobacter pylori.* Levofloxacin. Clarithromycin. Amoxicillin. Tetracycline. Metronidazole.

INTRODUCTION

Helicobacter pylori is thought to be involved in the pathogenesis of gastritis, peptic ulcer disease, gastric cancer, mucosa-associated lymphoid tissue lymphoma and various extra-gastrointestinal manifestations[1] [2] [3]. Unfortunately, emerging bacterial resistance causes treatment problems, leading to a search for new treatment regimens.

Helicobacter pylori antibiotic resistance is associated with both genetic alterations and biofilm formation[4] [5] [6]. A number of recent studies have claimed that sub-inhibitory antibiotic concentrations or impermeable environmental conditions in the body stimulate the transformation of *H. pylori* into a viable but nonculturable coccoid form. These circumstances occur more frequently in biofilms, causing bacterial cells to remain in their inactivated form, which is less susceptible to antibiotics [5] [6] [7].

A polymerase chain reaction (PCR) method has been developed for the detection of clarithromycin resistance[8]. This technique detects clarithromycin resistance-conferring mutations in the peptidyl transferase region, which is encoded within domain V of the *H. pylori* 23S ribosomal ribonucleic acid (RNA) gene. The A2143G, A2142G and A2142C mutations are responsible for 90% of the cases of primary clarithromycin resistance in western countries[9]. The major reason for *H. pylori* infection treatment failure is resistance to one or more of the antibiotics used for treatment. Resistance to clarithromycin or metronidazole has a negative impact on treatment outcome. However, the effect of clarithromycin resistance on the success of bacterial eradication is more prominent than that

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of metronidazole resistance[10].With respect to *H. pylori* resistance surveillance, many studies determining minimum inhibitory concentration (MIC) levels or resistance genes have been published from different geographic regions. Some national studies have reported the clarithromycin resistance of *H. pylori* strains, but few data about metronidazole and levofloxacin resistance are available, especially for northwestern Turkey[2] [4] [11]. The prevalence of clarithromycin-resistant *H. pylori* isolates is increasing over time, and their prevalence is higher in developing countries than in developed countries[12]. Therefore, to apply effective treatment, it is particularly important to know the antibiotic resistance patterns of local *H. pylori* strains, particularly in developing countries such as Turkey. Levofloxacin, a fluoroquinolone, has remarkable in vitro activity against *H. pylori*, and it has been mainly evaluated as a second-line therapy for use after one or more *H. pylori* eradication failures. Unfortunately, a progressive increase in the *in vitro* resistance of *H. pylori* to this quinolone has developed in recent years[3].

The aim of this study was to assess the prevalence of clarithromycin, amoxicillin, tetracycline, and metronidazole resistance and also to assess the prevalence of levofloxacin resistance among *H. pylori* strains isolated from Turkish patients with dyspepsia in northwestern Turkey. In light of our findings, we hope to guide the development of an *H. pylori* eradication program in this region that takes resistance rates into account.

METHODS

Study design

The present work was designed as a prospective randomized study between February 2012 and January 2013. Ninety-eight patients with dyspeptic symptoms who were admitted to the endoscopy unit of Istanbul University in the Cerrahpasa Faculty of Medicine in the Department of Internal Medicine and the Division of Gastroenterology were enrolled during this period. The inclusion criterion was the indication of endoscopy for the examination of dyspeptic symptoms. The exclusion criteria were as follows: age under 18; previous gastric surgery and *H. pylori* eradication treatment; consumption of antibiotics in the previous month; or consumption of antisecretory drugs, bismuth salts, or sucralfate in the previous 2 weeks. A history of bleeding or a coagulation disorder that contraindicated biopsy sampling were also reasons for exclusion. Two biopsies from each subject, from the antrum and the corpus of the stomach, were obtained. The biopsies were separately transferred into sterile Eppendorf tubes filled with *Brucella* broth containing 20% glucose and were then immediately sent to the laboratory on dry ice.

Helicobacter pylori isolation and antimicrobial susceptibility testing

Helicobacter pylori culture was performed using Columbia agar plates supplemented with 10% (v/v) defibrinated horse blood and an *H. pylori*-selective antibiotic supplement (Becton Dickinson GmbH, Heidelberg, Germany) containing vancomycin (10mg/L), cefsulodin (5mg/L), trimethoprim (5mg/L) and amphotericin B (5mg/L). The plates were incubated for 3-5 days at 37°C in a Campy Gen Gas Pak (Becton Dickinson). *Helicobacter pylori* was identified based on colony and cellular morphology (i.e., Gram-negative and helix-shaped under the microscope, forming circular convex and translucent colonies) and based on positive urease, catalase and oxidase tests. Some of the colonies were stored in *Brucella* broth containing 20% glycerol at -80°C[13]. Duplicate biopsy materials from the corpus and antrum were stored in PBS at -80°C in Eppendorf tubes for the detection of clarithromycin resistance via PCR.

The antimicrobial susceptibility of the *H. pylori* isolates was determined using the E-test (BioMerieux, Marci L'étoile France) on Mueller-Hinton agar supplemented with 10% horse blood. The *H. pylori* strain ATCC 43504 was used as a standard control. The **Table 1** shows that resistance of all isolates was determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[14].

Detection of clarithromycin susceptibility using PCR

Biopsies from the antrum and corpus were homogenized using a Magna Lyser Homogenizer (Roche Diagnostic, Basel, Switzerland) and genomic deoxyribonucleic acid (DNA) was extracted using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions;the isolated genomic DNA was stored at -20°C. In clarithromycin-resistant strains, point mutations in the 23S

TABLE 1 - The EUCAST[14] (2014) breakpoints used for *Helicobacter pylori* isolates.

	MIC (mg/L)	
	susceptible ≤	resistant ≥
Amoxicillin	0.12	0.12
Clarithromycin	0.25	0.5
Tetracycline	1	1
Metronidazole	8	8
Levofloxacin	1	1

MIC: minimal inhibitory concentration. **EUCAST:** European Committee on Antimicrobial Susceptibility Testing.

ribosomal ribonucleic acid (rRNA) gene were investigated using the *H. pylori* & Clarithromycin Resistance Real-Time PCR Kit (AndiaTec GmbH, Kornwestheim, Germany). In this method, the *H. pylori* 23S rRNA gene sequence was amplified, and the melting curve of the amplicon was then analyzed using real-time PCR. Thus, clarithromycin-resistant strains were determined based on the detection of A2143G and A2144G mutations. PCR and hybridization reactions were conducted using a Light Cycler 2.0 (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions.

Real-time PCR amplification consisted of an initial denaturation step at 95°C for 10 min that was followed by 45 amplification cycles (with a temperature transition rate of 20°C/s) consisting of 95°C for 0 s, annealing at 55°C for 10 s, and extension at 72°C for 15 s. After amplification, a melting step was performed, which consisted of 95°C for 0 s, cooling to 55°C for 5 s (with a temperature transition rate of 20°C/s), and finally a slow rise in the temperature to 80°C at a rate of 0.1°C/s, with continuous acquisition of the fluorescence decline.

Ethical considerations

The study was approved by the Clinical Research Ethics Board of Istanbul University, Cerrahpasa Faculty of Medicine, and all patients gave their informed consent before participating in the study. Ethical approval: 4548/9 February 2012.

RESULTS

A total of 98 *H. pylori* strains were isolated from the patients. The mean patient age was 46 (range, 20-70); 57% (n=56) of the patients were female, and 43% (n=42) were male. According to the EUCAST breakpoints, all of the tested isolates were susceptible to amoxicillin (MIC range, <0.016 – 0.032mg/L) and tetracycline (MIC range, <0.016 – 0.032mg/L). However, the **Table 2** shows that clarithromycin resistance was observed in 36.7% (36/98) of the isolates (MIC range, <0.016 –> 256mg/L); metronidazole resistance was observed in 35.5% (34/98) of the isolates (MIC range, <0.016 –>256mg/L) and levofloxacin resistance was found in 29.5% (29/98) of the isolates (MIC range, <0.008 – 32mg/L).

Point mutations were detected at nucleotide positions 2143 (A2143G) and 2144 (A2144G) of the 23S rRNA gene in all of the 36 clarithromycin-resistant *H. pylori* strains. Multiple resistance was detected in 19.3% of the total isolates and in 52.7% (19/36) of the 36 clarithromycin-resistant isolates. The most frequent multiple resistance phenotypes were clarithromycin/metronidazole and clarithromycin/levofloxacin, with rates of 45.4% and 27.2%, respectively. Additionally, the prevalence of clarithromycin/metronidazole/levofloxacin multiple resistance was 18.1%.

DISCUSSION

The success rate of standard clarithromycin-amoxicillin-proton pump inhibitor (PPI) triple therapy has declined to less than 70% from an initial rate of 90%. Resistance to clarithromycin, which is the key antibiotic in triple therapies for *H. pylori* infections, is primarily responsible for this decrease[15]. As an alternative to clarithromycin, new second-line treatment regimens that contain levofloxacin have been disappointing due to emerging levofloxacin resistance[2][3][4]. As in the case of the high clarithromycin resistance rate in Turkey, a sequential regimen is currently used, namely a simple dual therapy of PPI 2x1 + amoxicillin 1g 2x1 (first 7 days) and PPI 2x1, tetracycline 500mg 4x1 + metronidazole 500mg 3x1 (for the remaining 7 days), for the eradication of *H. pylori* infections[16].

High clarithromycin and metronidazole resistance rates have been reported in most studies. For example, among countries neighboring Northwestern Turkey, the clarithromycin and metronidazole resistance rates in untreated patients in Greece were reported to be 31% and 43%, respectively, by Georgopoulos et al.[17]; in Bulgaria, the corresponding rates were and 20.8% and 31.2%, as reported by Boyanova et al.[18] **(Table 3)**. In a 2008-2009 multicenter study that included 32 centers in 18 European countries, the *H. pylori* clarithromycin resistance rates were 21.5%, 18.7% and 7.7% in Southern Europe, central Europe and Northern Europe, respectively. The metronidazole resistance rates were 29.7%, 43.8% and 28.6% for the same regions[19]. According to this multicenter study, the *H. pylori* resistance rates to clarithromycin and levofloxacin were significantly higher in Western/central and Southern Europe

TABLE 2 - Antimicrobial susceptibilities of 98 *Helicobacter pylori* isolates.

Antibiotic	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Susceptible		Resistant	
				n	%	n	%
Amoxicillin	<0.016 - 0.032	0.016	0.032	98	100.0	0	0.0
Tetracycline	<0.016 - 0.032	0.016	0.032	98	100.0	0	0.0
Clarithromycin	<0.016 - >256	0.016	256	62	63.2	36	36.7
Metronidazole	<0.016 - >256	0.250	256	64	64.5	34	35.5
Levofloxacin	<0.008 - >32	0.060	<32	69	70.4	29	29.5

MIC: minimal inhibitory concentration.

TABLE 3 - Distributions of amoxicillin, clarithromycin, metronidazole, tetracycline and levofloxacin resistance rates in *Helicobacter pylori* isolates from different regions of Turkey and neighboring countries.

Region of Turkey Years	AMO (%)	CLA (%)	Method	MTZ (%)	TET (%)	LEV (%)	Reference
Central Turkey							
2012	0	28.5	ET	39.2	-	-	11
Southern Turkey							
2013	-	8.8	PCR-RFLP/AD	-	-	-	28
2012	0	18.2	ET	45.5	9.1	18.2	4
Northwestern Turkey							
2014	0	36.7	ET/PCR*	35.5	0	29.5	Present study
2009	3.2	41.9	ET/FISH*	41.9	3.2	-	29
Western Neighbor Countries							
2014/Bulgaria	0	20.8	ET/BST	31.2	2.1	16.7	18
2013/Greece	0	31	ET	43.7	0	10	17

AMO: amoxicillin; **CLA:** clarithromycin; **MTZ:** metronidazole; **TET:** tetracycline; **LEV:** levofloxacin; **ET:** E-test; **PCR-RFLP:** polymerase chain reaction-restriction fragment length polymorphism; **AD:** agar dilution; **FISH:** fluorescence *in situ* hybridization; **BST:** breakpoint susceptibility test. *****Method used only for the determination of clarithromycin resistance.

(>20%) than in Northern European countries (<10%)[19]. The main results of the multicenter survey showed a steady increase in clarithromycin resistance when compared to a similar 1998 survey and revealed the rapid emergence of levofloxacin resistance in *H pylori*. It also reported that the prevalence of clarithromycin resistance almost doubled over the past 10 years, from 9.8% to 17.5%, but that metronidazole resistance remained at the same high level (34.9%) over the past decade, with no major changes in regional distribution. Megraud et al.[19] suggested that the impact of metronidazole resistance on the eradication rate is limited relative to clarithromycin and levofloxacin resistance and that metronidazole resistance can be overcome in a majority of cases by increasing the length of treatment or by prescribing bismuth-containing quadruple therapy that includes metronidazole. Similarly high clarithromycin resistance rates have been reported in Italy and Germany, at 53% and 67.1%, respectively[9] [20]. A contrasting example is Norway, where macrolides are rarely selected as a treatment option; there, the prevalence of clarithromycin resistance was reported to be 5.9%[21]. In East Asian countries such as Japan (27.2%), South Korea (23.7%) and China (65.4%), high clarithromycin resistance rates have been reported. In Southeast Asian countries, clarithromycin resistance rates are low, as in Thailand (3.7%) and Singapore (6%). Moreover, in Bhutan and Malaysia, no clarithromycin resistance has been reported [22].

The overall clarithromycin resistance prevalence in Latin America (13%) is below 15-20%, and the rates range from 8%-18% in Brazil. Peru (50%) reports the highest prevalence, whereas Paraguay (2%) reports the lowest. Camargo et al.[23] suggest in their meta-analysis that the empirical use of clarithromycin may be inappropriate in Peru and Colombia[23]. In Brazil, *H. pylori* clarithromycin resistance shows high prevalence, with rates of 7-16% in adults and 27% in children[24]. The metronidazole resistance of *H. pylori* is closely related to geographic location and to the use of this drug for its antiparasitic activity. In African countries, the prevalence of metronidazole resistance is approximately 90%; however, this rate decreases to 45% in Spain and 22.5% in Norway[19] [21] [25] [26] [27].

In East Asia, China has the highest prevalence of metronidazole resistance (56.6%-95.4%), and metronidazole resistance is also high in Hong Kong and South Korea. However, the metronidazole resistance rate in Japan is only 3.3%-4.9%. In Southeast Asia, only Thailand and Malaysia have metronidazole resistance rates below 40%. Bhutan (82.9%) and Indonesia (100%) have the highest metronidazole resistance in this region. High prevalences of metronidazole resistance have also been reported in western and southern Asia[22]. Camargo et al.[23] found that metronidazole resistance in Latin American populations was high and stable over the study period and showed no remarkable trends within individual countries, with the lowest resistance rate occurring in Argentina (30%) and the highest in Colombia (83%). The metronidazole resistance rate is 54% in Brazil, but in contrast to clarithromycin resistance, metronidazole resistance is not of great clinical relevance[23]. The clarithromycin resistance rates are 41% and 28.5% in Northwestern and Central Turkey, respectively, and were 18.2% in 2012 and 8.9% in 2013 in Southern Turkey[4] [11] [28]. On the other hand, metronidazole resistance rates were 39.2% and 45.5% in central and Southern Turkey, respectively[4] [11]; in Northwestern Turkey, the rate was 41.9% in 2009[29] **(Table 3)**. Based on these data, the Turkish clarithromycin resistance rate (36.7%) was higher than that the rates in Latin America (except for Peru), in East Asian countries (except China)

and in the above studies (except Italy and Germany), and it was only lower than the rate in northwestern Turkey. Clarithromycin resistance is commonly caused by point mutations in the 23S rRNA gene of *H. pylori*[9]. In Turkey, elderly patients are more likely to have respiratory tract infections, for which macrolides are commonly prescribed. Previous macrolide use may have caused clarithromycin resistance in the *H. pylori* strains analyzed in our study. Our observed metronidazole resistance rate (35.5%) was higher than that in Norway, southern Europe and northern Europe but lower than that in Latin America, Western and Southern Asia, African countries, China, Spain, Brazil and Central Europe and also lower than that in Central, Southern and Northwestern Turkey. The high metronidazole resistance rates in other regions might be due to the common use of drugs for parasitic infestations in these areas.

After detecting a high clarithromycin resistance rate among our *H. pylori* strains, we used a real-time PCR kit to detect point mutations in the 23S rRNA gene of our clarithromycin-resistant *H. pylori* strains to understand the etiology of their clarithromycin resistance. International and national studies of point mutations related to clarithromycin resistance have been published. Momynaliev et al.[30] reported that in 10 analyzed clarithromycin-resistant clinical isolates of *H. pylori* obtained in Russia, resistance was found to be mediated solely by the A2144G mutation in the 23S rRNA gene. The prevalence of each point mutation varies according to geographical area. Versalovic et al.[31] and Raymond et al.[32] reported 52.5% and 90% A2143G (previously A2144G) mutations among American and French *H. pylori* isolates, respectively. Alvarez et al.[33] also confirmed the existence of A2143G mutations among patients from Colombia. In a review by Mégraud et al.[19] the most common mutation was A2143G, which occurred in approximately 69.8% of clarithromycin-resistant strains. The A2143G mutation was found in 42.9% of clarithromycin-resistant *H. pylori* strains by Klesiewicz et al.[34]. In Turkey, Yula et al.[28] and Sezgin et al.[35] found A2143G mutations in 6.6% and 40.5% of *H. pylori* strains, respectively. However, we detected point mutations at nucleotide positions 2143 (A2143G) and 2144 (A2144G) of the 23S rRNA gene in 100% of our *H. pylori* strains. A limitation of our study is that the real-time PCR kit we used, does not discriminate between point mutations at nucleotide positions 2143 (A2143G) and 2,144 (A2144G) of the 23S rRNA gene in *H. pylori* strains.

When we evaluated two other antibiotics (amoxicillin and tetracycline) used for the treatment of *H. pylori* infections, we found lower resistance rates. In 2013, Megraud et al.[19] reported 0.7% and 0.9% amoxicillin and tetracycline resistance rates, respectively. Wueppenhorstet al.[20] and Larsen et al.[21] in Norway reported *H. pylori* amoxicillin and tetracycline resistance rates of 1.4% and 0% in Germany and 10% and 0% in Norway, respectively. Cuadrado-Lavínet al.[27] in Spain and Georgopoulos et al.[17] in Greece reported no amoxicillin resistance. Boyanova et al.[18] reported 2% tetracycline resistance. Most studies have indicated that the rates of resistance to amoxicillin are < 1% in China, Bahrain, Malaysia, Bhutan and Vietnam but are >10% in Japan. Higher amoxicillin resistance rates have also been detected in South Korea (7.1%-18.5%). India (72.5%) and Pakistan (37%) also have high rates of amoxicillin resistance. In Southeast Asia, only Indonesia (19.4%) has high resistance rates[22]. The relatively low overall prevalence of amoxicillin resistance in Latin America is similar to that of other regions. Amoxicillin resistance rates range from 0-15% in Latin America and are 2% in Brazil. Thus, the inclusion of this antibiotic in empirical eradication regimens is still appropriate worldwide[23]. Zhang et al.[36] reported that *H. pylori* isolates were relatively susceptible to tetracycline, with an overall resistance rate of 4.9%-7.3%, thus suggesting that tetracycline can be used in the initial treatment of *H. pylori* infections in China. Resistance to tetracycline is very low, or even absent, in most Asian countries. Resistance rates in Saudi Arabia, Thailand, and Vietnam are also reportedly low, and resistance is absent in Taiwan, Bahrain, Malaysia, and Bhutan. In contrast, higher values have been reported in South Asia, for example in South Korea (35.2%)[22]. Primary resistance to tetracycline was evaluated in 20 Latin American studies, and the study-specific prevalence values ranged from 0% to 86%. The overall prevalence was 6%. The highest resistance was reported in Colombia, and 2% resistance was reported in Brazil[23]. Similar to the rates reported for Central Europe, the amoxicillin resistance rate was 0% in Central and Southern Turkey. In contrast, in Northwestern Turkey, the amoxicillin resistance rate was reported to be 3.2% in 2009[29]. In Southern and Northwestern Turkey, the tetracycline resistance rates were reported to be 9.1% and 3.2%, respectively[4] [29]. In contrast, in the present study, which was performed in Istanbul, no tetracycline resistance was detected. We detected no amoxicillin or tetracycline resistance in our study, and our results are in accord with those of Georgopoulos et al.[17] and Boyanova et al.[18] for amoxicillin resistance.

Although the eradication success of the new regimen containing levofloxacin is as high as 90%, emerging studies are reporting increasing quinolone resistance rates[27] [37] [38]. In Europe, levofloxacin resistance rates have reached 18%[19] [21]. Karczewska et al.[39] reported levofloxacin resistance rates of 12% and 38% in treatment-naïve and previously levofloxacin-treated patients, respectively, in 2012. The rate of resistance to levofloxacin was reported to be 18.8% in Germany in 2013[20]. Fluoroquinolone resistance rates are low (8.8%) in Taiwan, but they are higher in the southeast coastal region of China and in Beijing. The levofloxacin and moxifloxacin resistance rates in South Korea increased to 34.6% over the 2009-2012 period. In Western and Southern Asia, strains show low levofloxacin resistance rates, whereas high levofloxacin and ciprofloxacin resistance rates have been reported in Iran (72.5%) and India (50%). The levofloxacin resistance rates in Southeast Asia are otherwise low except in Vietnam (18.4%)[22]. The overall prevalence of fluoroquinolone resistance in Latin America is higher than the overall levofloxacin resistance rates reported for Europe (14.1%) and Asia (11.6%)[23]. Eisig et al.[40] reported a levofloxacin resistance rate of 23% in Brazil. In Turkey, Cagdas et al.[4] reported that the levofloxacin resistance rate in southern Turkey was 18.2% in 2012 using a levofloxacin breakpoint MIC value of ≥ 2mg/L (they did not use the EUCAST 2013 breakpoint recommendation of ≥ 1mg/L).We determined the

prevalence of levofloxacin resistance to be 29.5%, and the MIC_{90} value was > 32mg/L (breakpoint MIC ≥ 1mg/L). Our result is higher than the results of Georgopoulos et al.[17] (16%) and Boyanova et al.[18] (10%) for levofloxacin resistance rates. This difference could be due to the crowded population, to the geographic location of Istanbul or to the frequent use of this antibiotic for respiratory and/or urinary infections in this area.

However, we believe that levofloxacin resistance rates might be increasing due to more frequent use of quinolones. There has been an increasing trend in levofloxacin resistance over time, and after eradication failures with levofloxacin, multiple resistant *H. pylori* strains have gained importance. Boyanova et al.[41] reported an 8% metronidazole/clarithromycin resistance rate in *H. pylori* strains. In a study performed in 2012 in Spain, the authors reported an 18.1% multidrug resistance rate, with the metronidazole/quinolone resistant phenotype as the predominant phenotype[3]. In the present study, multiple resistance was observed in 52.7% (19/36) of the 36 clarithromycin-resistant strains. The most common dual-resistance phenotypes were clarithromycin/metronidazole (45.4%) and clarithromycin/levofloxacin (27.2%). In addition, 18.1% of the study strains were found to harbor clarithromycin/metronidazole/levofloxacin multidrug resistance.

Our study is the first study in Turkey to report levofloxacin MIC values of > 32mg/L in *H. pylori* and to report 27.2% levofloxacin resistance rates in clarithromycin-resistant strains using the EUCAST[14] breakpoint (≥ 1mg/L) recommendations. As a result, the resistance rates we detected for clarithromycin (36.7%), metronidazole (35.5%) and levofloxacin (29.5%) are higher and may be associated with treatment failures in Turkish patients. To effectively treat *H. pylori* infections, it is particularly important to know the antibiotic resistance patterns of local *H. pylori* strains. The creation of an *H. pylori* resistance map using actual data from different regions of the world is crucial for the development of appropriate eradication treatment alternatives. Without new treatment alternatives, we believe that *H. pylori* eradication may become an important problem in our country in the future. The increasing antimicrobial resistance of *H. pylori* and the emergence of multidrug-resistant strains are important issues that require the identification of new, more effective treatment alternatives.

CONFLICT OF INTEREST

I declare on behalf of all authors that there is no conflict of interest related to this manuscript. This study was presented as a poster presentation at the 'XXVIth International Workshop on *Helicobacter* and related bacteria in chronic digestive inflammation and gastric cancer from September 12-14, 2013, in Madrid, Spain (P 13.18 and P 13.20).

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REFERENCES

1. Georgopoulos SD, Papastergiou V, Karatapanis S. Current options for the treatment of *Helicobacter pylori*. Expert Opin Pharmacother 2013; 14:211-223.
2. Aydin A, Oruc N, Turan I, Ozutemiz O, Tuncyurek M, Musoglu A. The modified sequential treatment regimen containing levofloxacin for *Helicobacter pylori* eradication in Turkey. Helicobacter 2012; 14:520-524.
3. Cuadrado-Lavín A, Salcines-Caviedes JR, Carrascosa MF, Dierssen-Sotos T, Cobo M, Campos MR, et al. Levofloxacin versus clarithromycin in a 10 day triple therapy regimen for first-line *Helicobacter pylori* eradication: a single-blind randomized clinical trial. J Antimicrob Chemother 2012; 67: 2254-2259.
4. Cagdas U, Otag F, Tezcan S, Sezgin O, Aslan G, Emekdas G. Detection of *Helicobacter pylori* and antimicrobial resistance in gastric biopsy specimens. Mikrobiyol Bul 2012; 46:398-409.
5. Cammarota G, Sanguinetti M, Gallo A, Posteraro B. Review article: biofilm formation by *Helicobacter pylori* as a target for eradication of resistant infection. Aliment Pharmacol Ther 2012; 36:222-230.
6. Cole SP, Harwood J, Lee R, She R, Guiney DG. Characterization of monospecies biofilm formation by *Helicobacter pylori*. J Bacteriol 2004; 186:3124-3132.
7. Carron MA, Tran VR, Sugawa C, Coticchia JM. Identification of *Helicobacter pylori* biofilms in human gastric mucosa. J Gastrointest Surg 2006; 10:712-717.
8. de Francesco V, Margiotta M, Zullo A, Hassan C, Valle ND, Burattini O, et al. Primary clarithromycin resistance in Italy assessed on *Helicobacter pylori* DNA sequences by TaqMan real-time polymerase chain reaction. Aliment Pharmacol Ther 2006; 23:429-435.
9. Mono R, Giorgio F, Carmine P, Soleo L, Cinquepalmi V, Ierardi E. *Helicobacter pylori* clarithromycin resistance detected by Etest and TaqMan real-time polymerase chain reaction: a comparative study. APMIS 2012; 120:712-717.
10. Dore MP, Leandro G, Realdi G, Sepulveda AR, Graham DY. Effect of pre-treatment antibiotic resistance to metronidazole and clarithromycin on outcome of *H. pylori* therapy: a meta-analytical approach. Dig Dis Sci 2000; 45: 68-76.
11. Kalem F, Ozdemir M, Basaranoglu M, Toy H, Baysal B. *Helicobacter pylori* isolates recovered from antral gastric biopsies of patients with dyspeptic symptoms: Antimicrobial resistance of metronidazole, clarithromycin and amoxicillin. Anatol J Clin Investig 2012; 6:37-40.
12. Hu C, Wu C, Lin C, Cheng CC, Su SC, Tseng YH, et al. Resistance rate to antibiotics of *Helicobacter pylori* isolates in eastern Taiwan. J Gastroenterol Hepatol 2007; 22:720-733.
13. Ansorg R, von Recklinghausen G, Pomarius R, Schmid EN. Evaluation of techniques for isolation, subcultivation, and preservation of *Helicobacter pylori*. J Clin Microbiol 1991; 29:51-53.
14. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 3.1. 2014. (Cited 2015 January 26). Available at http://www.eucast.org
15. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. Gut 2010; 59:1143-1153.
16. Şimşek İ, Binicier ÖB. *Helicobacter pylori*. İç Hastalıkları Dergisi. Turkish J Int Med 2011; 18:13-26. (In Turkish).
17. Georgopoulos SD, Xirouchakis E, Martinez-Gonzalez B, Sgouras DN, Spiliadi C, Mentis AF, et al. Clinical evaluation of a ten-day regimen with esomeprazole, metronidazole, amoxicillin, and

clarithromycin for the eradication of *Helicobacter pylori* in a high clarithromycin resistance area. Helicobacter 2013; 18:459-467.

18. Boyanova L, Davidkov L, Gergova G, Kandilarov N, Evstatiev I, Panteleeva E, et al. *Helicobacter pylori* susceptibility to fosfomycin, rifampin, and 5 usual antibiotics for *H. pylori* eradication. Diagn Microbiol Infect Dis 2014; 79:358-361.
19. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. Study Group participants: *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut 2013; 62**:**34-42.
20. Wueppenhorst N, Stueger HP, Kist M, Glocker EO. High secondary resistance to quinolones in German *Helicobacter pylori* clinical isolates. J. Antimicrob Chemother 2013; 68:1562-1566.
21. Larsen AL, Ragnhildstveit E, Moayeri B, Eliassen L, Melby KK. Resistance rates of metronidazole and other antibacterials in *Helicobacter pylori* from previously untreated patients in Norway. APMIS 2013; 121:353-358.
22. Miftahussurur M, Yamaoka Y. Appropriate First-Line Regimens to Combat *Helicobacter pylori* Antibiotic Resistance: An Asian Perspective. Molecules 2015; 20:6068-6092.
23. Camargo MC, García A, Riquelme A, Otero W, Camargo CA, Hernandez-García T, et al. The problem of *Helicobacter pylori* resistance to antibiotics: a systematic review in Latin America. Am J Gastroenterol 2014; 109:485-495.
24. Suzuki RB, Lopes RA, da Câmara Lopes GA, Hung Ho T, Sperança MA. Low *Helicobacter pylori* primary resistance to clarithromycin in gastric biopsy specimens from dyspeptic patients of a city in the interior of São Paulo, Brazil. BMC Gastroenterol 2013; 13:164.
25. Francesco V, Zullo A, Hassan C, Giorgio F, Rosania R, Ierardi E. Mechanisms of *Helicobacter pylori* antibiotic resistance: an updated appraisal. World J Gastrointest Pathophysiol 2011; 2:35-41.
26. de Francesco V, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, et al. Worldwide *Helicobacter pylori* antibiotic resistance: a systematic review. J Gastrointestin Liver Dis 2010; 19:409-414.
27. Cuadrado-Lavín A, Salcines-Caviedes JR, Carrascosa MF, Mellado P, Monteagudo I, Llorca J, et al. Antimicrobial susceptibility of *Helicobacter pylori* to six antibiotics currently used in Spain. J Antimicrob Chemother 2012; 67:170-173.
28. Yula E, Nagiyev T, Kaya OA, Inci M, Celik MM, Köksal F. Detection of primary clarithromycin resistance of *Helicobacter pylori* and association between cagA (+) and clinical outcome. Folia Microbiol (Praha) 2013; 58:141-146.
29. Ozbey SB, Cuneyt O, Murat K. Antibiotic resistance rates of *Helicobacter pylori* isolates and the comparison of E-Test and fluorescent in situ hybridization methods for the detection of clarithromycin resistant strains. Mikrobiyol Bul 2009; 43:227-234.
30. Momynaliev KT, Selezneva OV, Kozlova AA, Vereshchagin VA, Il'ina E, Govorun VM. A2144G is the main mutation in the 23S rRNA gene of *Helicobacter pylori* associated with clarithromycin resistance. Genetika 2005; 41:1338-1344.
31. Versalovic J, Osato MS, Spakovsky K, Dore MP, Reddy R, Stone GG, et al. Point mutations in the 23S rRNA gene of *Helicobacter pylori* associated with different levels of clarithromycin resistance. J Antimicrob Chemother 1997; 40**:**283-286.
32. Raymond J, Burucoa C, Pietrini O, Bergeret M, Decoster A, Wann A, et al. Clarithromycin resistance in *Helicobacter pylori* strains isolated from French children: prevalence of the different mutations and coexistence of clones harboring two different mutations in the same biopsy. Helicobacter 2007; 12:157-163.
33. Alvarez A, Moncayo JI, Santacruz JJ, Santacoloma M, Corredor LF, Reinosa E. Antimicrobial susceptibility and mutations involved in clarithromycin resistance in *Helicobacter pylori* isolates from patients in the western central region of Colombia. Antimicrob Agents Chemother 2009; 53:4022-4024.
34. Klesiewicz K, Nowak P, Karczewska E, Skiba I, Wojtas-Bonior I, Sito E, et al. PCR-RFLP detection of point mutations A2143G and A2142G in 23S rRNA gene conferring resistance to clarithromycin in *Helicobacter pylori* strains. Acta Biochim Pol 2014; 61:311-315.
35. Sezgin O, Aslan G, Altintaş E, Tezcan S, Serin MS, Emekdaş G. Detection of point mutations on 23S rRNA of *Helicobacter pylori* and resistance to clarithromycin with PCR-RFLP in gastric biopsy specimens in Mersin, Turkey. Turk J Gastroenterol 2008; 19:163-167.
36. Zhang YX, Zhou LY, Song ZQ, Zhang JZ, He LH, Ding Y. Primary antibiotic resistance of *Helicobacter pylori* strains isolated from patients with dyspeptic symptoms in Beijing: A prospective serial study. World J Gastroenterol 2015; 21:2786-2792.
37. O'Connor A, Gisbert J, O'Morain C. Treatment of *Helicobacter pylori* infection. Helicobacter 2009; 14: 46-51.
38. Glocker E, Stueger HP, Kist M. Quinolone resistance in *Helicobacter pylori* isolates in Germany. Antimicrob Agents Chemother 2007; 51:346-349.
39. Karczewska E, Klesiewicz K, Skiba I, Wojtas-Bonior I, Sito E, Czajecki K, et al. Variability in prevalence of *Helicobacter pylori* strains resistant to clarithromycin and levofloxacin in southern Poland. Gastroenterol Res Pract 2012; 2012:418010.
40. Eisig JN, Silva FM, Barbuti RC, Navarro-Rodriguez T, Moraes-Filho JP, Pedrazzoli Jr J. *Helicobacter pylori* antibiotic resistance in Brazil: clarithromycin is still a good option. Arq Gastroenterol 2011; 48:261-264.
41. Boyanova L. Prevalence of multidrug-resistant *Helicobacter pylori* in Bulgaria. J Med Microbiol 2009; 58:930-935.