Observational Study

Medicine

OPEN

Recent trends in the antibiotic resistance of *Helicobacter pylori* in patient with dyspepsia

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Abstract

The aim of this study was to determine the resistance status and to identify the point mutations conferring resistance to clarithromycin and fluoroquinolones among dyspeptic patients in Manisa, Turkey.

The study included a sample of 140 patients with an indication for upper gastrointestinal endoscopy randomly selected from 2100 dyspeptic patients attending to the Gastroenterology and Endoscopy Unit at Manisa Celal Bayar University Hafsa Sultan Hospital between April 2016 and May 2018. A commercially available GenoType Helico DR test was used to detect the presence of *Helicobacter pylori* and mutations associated with resistance to clarithromycin and fluoroquinolones in biopsy specimens.

In total, 116 (82.9%) of 140 biopsies obtained from the same number of dyspeptic patients were positive for *H pylori* and 82 (approximately 71%) of them harbored resistance mutations in 23SrRNA and/or gyrA. Resistance to clarithromycin, levofloxacin, or both were detected in 43.1% (50/116), 27.6% (32/116), and 16/116 (13.8%) of tested biopsies, respectively. The most common mutation conferring resistance to clarithromycin was A2147G (96%, 48/50). Resistance to fluoroquinolones was frequently due to mutation in codon 91 and the most common mutation detected was D91G (34.4%). Heteroresistance patterns were observed in 48.0% (24/50) of clarithromycin-resistant samples and 28.1% (9/32) of levofloxacin-resistant samples.

The resistance rates and detected mutations in this study are in line with the country data. However, to achieve better *H pylori* eradication and to prevent the spread of multidrug-resistant strains in Turkey, the molecular-based susceptibility tests should be considered routinely. Further studies are needed to determine the various mutations among resistant strains.

Abbreviations: CI = confidence interval, OR = odds ratios, Ref = reference, WT = wild-type.

Keywords: antimicrobial resistance, clarithromycin, *Helicobacter pylori*, levofloxacine

1. Introduction

Today, it is well known that the major negative impact on the results of *Helicobacter pylori* eradication therapies is the increase in antibiotic resistance, especially clarithromycin and quinolones.[1–4] The resistance arises as consequence of point mutations in bacterial DNA, and the point mutations at positions 2146 and 2147 (formerly know as 2142 and 2143) in 23S rRNA gene, and the mutations at codons 87 and 91 of gyrA gene have been described as causeful for over 80% to 90% of clarithromycin and quinolone resistance cases, respectively.[5,6]

There are several experimented molecular-based diagnostic kits for detection of *H pylori* and its antibiotic resistance The molecular test have several advantages to conventional culture and culture-based antibiotic susceptibility tests.[7] GenoTypeHelicoDR (Hain Lifescience, Nehren, Germany) is a rapid molecular test for simultaneous detection of *H pylori* and its resistance to clarithromycin and quinolone based on DNA strip technology. Test including multiplex polymerase chain reaction amplification with subsequent reverse hybridization step. The strips were designed to identify the mutations, A2146C, A2146C, and A2147G for 23S rRNA gene and N87K, D91N, D91G, and D91Y for gyrA.

The clarithromycin resistance varies according to geographical areas, with higher percentages in some Asian countries such as Korea (60%), China (52.6%), and Japan (31.1%). Turkey also ranks among the countries with high *H pylori* clarithromycin resistance rates (40%), and consequently the eradication rates with standard triple therapy have reported as decreased to 55.7%.[2,4] According to earlier recent systematic review that included studies performed in Turkey between 1999 and 2015 found an overall prevalence of primary clarithromycin resistance of 24.8%, which varied regionally from 8.8% to 50%.[8]

In Turkey, quinolone-based protocols, mainly including levofloxacin, have been proposed in second- or third-line *H pylori* eradication therapies.[1] However, the effect of quinolone resistance rates on the effectiveness of *H pylori* eradication regimens in the country is unknown. In a study conducted in Mersin (in the Mediterranean region) in 2012, the quinolone resistance rate was reported to be 18.2% while the rate was 29.5% in Northwest Turkey in 2015.[9,10] In a recent study that used molecular methods, the rate of quinolone resistance was

Consent forms were obtained from all patients.

The authors have no funding and conflicts of interest to disclose.

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

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How to cite this article: Buran T, Sürücüoğlu S, Kurutepe S, Gazi H. Recent trends in the antibiotic resistance of Helicobacter pylori in patient with dyspepsia. Medicine 2022;101:26(e29801).

Received: 28 June 2021 / Received in final form: 16 May 2022 / Accepted: 26 May 2022

http://dx.doi.org/10.1097/MD.0000000000029801

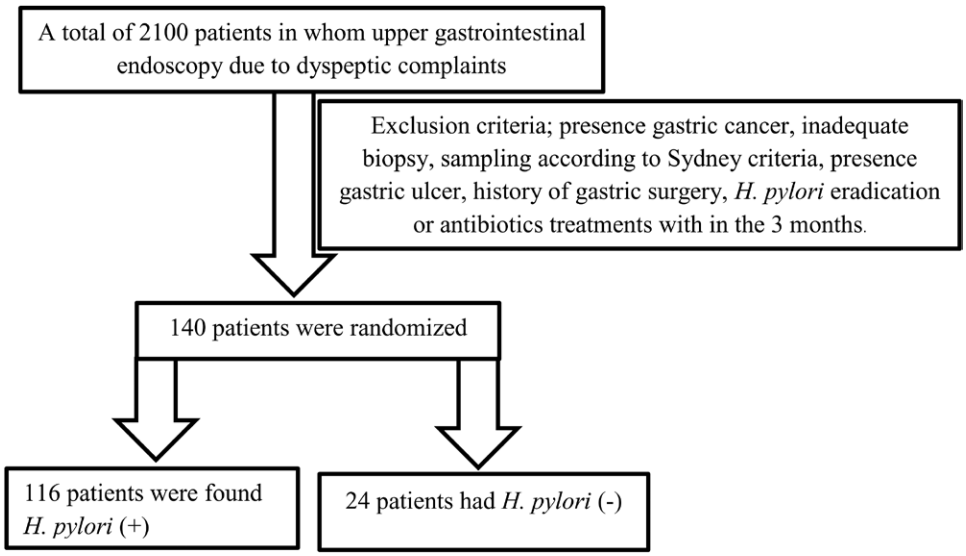

Figure 1. The study design.

reported to be 15% in the pediatrics population aged 2 to 18 years.[11] However, there is no previous report on antibiotic resistance and related mutations of *H pylori* among adult dyspeptic patients in Manisa.

In view of the importance of local resistance data for effective eradication of *H pylori*, the present study was carried out to determine the clarithromycin and quinolone resistance status and related mutations among dyspeptic patient in a tertiary care hospital in Manisa, Western Turkey.

2. Materials and methods

2.1. Patient selection and gastric biopsy sampling

This study included 140 subjects with dyspeptic disorders who were randomly selected from 2100 dyspeptic patients who attending to the Gastroenterology and Endoscopy Unit at Hafsa Sultan Hospital between April 2016 and May 2018. Patients were enrolled in the study based on clinical symptoms and positive endoscopic findings (such as gastritis and/or peptic ulcer) Patients with a history of an antibiotic in the previous 3 weeks and proton pump intake in the previous 3 months, and those aged younger than 18 years were excluded (Fig. 1).

Two antrum, 1 angular notch, and 2 corpus biopsies were taken from each patient. The first set of corpus, angular notch, and antrum biopsies were fixed and transported in 10% formalin solution for routine histopathological examination. The second set of biopsies was obtained only from patients who were eligible for inclusion in this study and were immediately transported to the Medical Microbiology Laboratory for molecular testing. The antrum, angular notch, and corpus samples obtained from each study participant were processed in a single container.

The study protocol was approved by the Clinical Research Ethics Committee of Manisa Celal Bayar University (study number: E-43401). Consent forms were obtained from all patients.

2.2. GenoType HelicoDR testing

Genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The extracted DNA was subsequently used for the molecular analyses. Polymerase chain reaction was performed with a thermal cycler (Applied Biosystems, Foster City, CA) using DNA polymerase (Hain Life Science GmbH, Nehren, Germany). The amplified DNA was added to biotinylated probes on the strips according to the manufacturer's instructions (Hain Life Science). 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 heteroresistance pattern.

2.3. Statistical analysis

A statistical software (SPPS 18.0, SPSS Inc. 2009. PASW Statistics for Windows, Version 18.0., Chicago, IL) was used for all the analyses. The association between resistance and demographic data was analyzed using logistic regression model. The odds ratios with 95% confidence intervals were calculated. For

Table 1

OR estimates for clarithromycin and levofloxacin resistance according to demographic data calculated with logistic regression.

	Clarithromycin resistant (n = 50)			Levofloxacin resistant (n = 32)		
Variables	**% (n)**	**OR (95% CI)**	***P***	**% (n)**	**OR (95% CI)**	***P***
Age						
≤50 (n = 56)	37.5 (21)	1.55 (0.74–3.27)	.240	28.6 (16)	0.90 (0.40–2.05)	.819
>50 (n = 60)	48.3 (29)	Ref		26.7 (16)	Ref	
Gender						
Female (n = 73)	41.1 (30)	0.80 (0.37–1.71)	.570	31.5 (23)	1.73 (0.71–4.21)	.221
Male (n = 43)	46.5 (20)	Ref		20.9 (9)	Ref	

CI = confidence interval, OR = odds ratio, Ref = reference.

Table 2

Genotypic susceptibility profiles of *Helicobacter pylori* detected in the study samples.

Antimicrobial	Susceptible (%)	Resistant (%)	Total (%)
Clarithromycin	66 (56.9)	50 (43.1)	116 (100)
Levofloxacin	84 (72.4)	32 (27.6)	116 (100)
Dual resistance (clarithromycin and levofloxacin)		16 (13.8)	116 (100)

statistical analyses, χ^2 test was used, and *P* values <.05 were considered statistically significant.

3. Results

One hundred sixteen (82.9%) of the 140 patients were *H pylori* positive, 37.1% (43/116) were male and 62.9% (73/116) were female. Mean age of the study subjects was 54.01 ± 1.52 years (Table 1). Clarithromycin resistance and levofloxacin resistance was observed in 43.1% (50/116) and 27.6% (32/116) of samples, respectively. Combined resistance to clarithromycin and levofloxacin was detected in 16 (13.8%) of the 116 specimens (Table 2).

The most common mutations detected in clarithromycin-resistant samples were A2147G.. The majority of gyrA mutations were observed at codon 91. The gyr87MUT (N87K) band was detected in 12.5% (4/32) of levofloxacin-resistant samples. A double mutation in codon 91 was observed in 6.3% (2/32) and in 12.5% (4/32) of patients (Table 3).

Heteroresistance (simultaneous presence of a WT band and a mutant band in the same sample) was identified in 48.0% (24/50) of the clarithromycin-resistant samples and 28.1% (9/32) of the fluoroquinolones-resistant samples.

4. Discussion

In this study, 43.1% of *H pylori* specimens were resistant to clarithromycin. This finding is compatible with the data from previous studies conducted in large cities in Turkey, including Ankara (50%), Izmir (48.2%), Bursa (41.9%), and Istanbul (41.9%) and indicate that this antibiotic has become unusable in the eradication of *H pylori* in Turkey.[8]

The A2147G (also known as A2143G) was the most common mutation (96%), as reported in many other studies, with a worldwide prevalence ranging from 53% to 95%.[3,6] In a recent Turkish study, the clarithromycin resistance rate among dyspeptic patients was reported to be 38.1% (24/63) and the most common found point mutation was A2143G (n = 11, 45.8%).[13] Also in the study, the higher minimum inhibitory concentrations were reported for the classical mutations (A2142G and A2143G) than for new identified mutations (A2115G, A2144T, G2141A), which indicates that the eradication rate is significantly reduced in the presence of the A2143G mutation than in the presence of other mutations.[13] Therefore, as in many previous studies, the high frequency of A2147G in our patients might be interpreted an important preliminary warning of eradication failure with clarithromycin.[3,13] However, more studies are needed to investigate the reflection of both classical and new mutations on the susceptibility test results as well as on therapeutic outcome.

The fluoroquinolone resistance rate of 27.6% in our region is compatible with the data reported from Northwestern Turkey (29.5%) and China (28%).[10,14] Higher fluoroquinolones resistance rates have been reported in countries with heavy quinolone use such as Nepal (42.9%) and Pakistan (62%).[15,16] Although there are no previous reports on the distribution of mutations related to quinolone resistance in Turkey, recent studies in other countries have identified mutations in codons 87 and 91 to be related to resistance in many cases.[3,8,17] The most common mutations detected in the present study were D91G (34%) and D91N (28.1%), which is similar to those detected with GenoType HelicoDR in Brazil (D91N, 34.8%; D91G, 18.1%).[5] Prescribing the initial eradication therapies based on local fluoroquinolone, susceptibility data will help to prevent the unfavorable impact of resistance on the success of quinolone-containing regimens as well as the probability of more resistant strains emerging under selection pressure.

In our study, the heteroresistance rates obtained in clarithromycin-resistant (48.0%) patients were similar but lower (28.1%) for levofloxacin obtained with Genotype Helico DR in southern Spain, 51.3% and 50%, respectively.[17] In a recent Turkish study, the antibiotic heteroresistance rate in a pediatric population was reported to be 17% (16/93); 12 mutations were determined in the 23S rRNA and 4 in the gyrA gene.[11] Further studies are needed to investigate the mutation patterns as well as the presence of heteroresistance and its clinical impact in *H pylori*-positive patients in Turkey.

Despite some design limitations (small sample size, missing clinical data, only 2 antimicrobials studied and only classical point mutations determined), the results of this study have both epidemiological and clinical importance for the area. Further epidemiological studies as well as close collaboration between clinicians and laboratories are needed to enhance the rate of successful eradication of *H pylori* in the country.

Table 3

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

Antimicrobial	Target gene	Samples with mutations detected	Type of mutation	n (%)
Clarithromycin	23S rRNA	50	23SMUT1 (A2146G)	2 (4)
			23SMUT2 (A2146C)	0
			23SMUT3 (A2147G)	48 (96)
Levofloxacin	gyrA	32	gyr87MUT (N87K)	4 (12.5)
			gyr91MUT1 (D91N)	9 (28.1)
			gyr91MUT2 (D91G)	11 (34.4)
			gyr91MUT3 (D91Y)	2 (6.3)
			gyr91MUT1 (D91N) gyr91MUT2 (D91G)	2 (6.3)
			gyr87MUT (N87K) gyr91MUT1 (D91N)	4 (12.5)
Dual resistance	23S rRNA plus gyrA	16	23SMUT1 (A2146G) gyr91MUT1 (D91Y)	1 (6.2)
			23SMUT3 (A2147G) gyr91MUT1 (D91G)	6 (37.5)
			23SMUT3 (A2147G) gyr91MUT2 (D91C)	5 (31.2)
			23SMUT3 (A2147G) gyr91MUT3 (D91Y)	1 (6.2)
			23SMUT3 (A2147G)gyr87MUT (N87K) gyr91MUT1 (D91N)	2 (12.5)
			23SMUT3 (A2147G)gyr91MUT1 (D91N) gyr91MUT2 (D91G)	1 (6.2)

Author contributions

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References

[1] Kaplan M, Tanoglu A, Duzenli T, et al. H. pylori treatment in Turkey: current status and rational treatment options. North Clin Istanb. 2019;7:87–94.

[2] Vilaichone RK, Quach DT, Yamaoka Y, et al. Prevalence and pattern of antibiotic resistant strains of H. pylori infection in ASEAN. Asian Pac J Cancer Prev. 2018;19:1411–13.

[3] Jaka H, Rüttgerodt N, Bohne W, et al. H. pylori mutations conferring resistance to fluoroquinoloness and clarithromycin among dyspeptic patients attending a tertiary hospital, Tanzania. Can J Gastroenterol Hepatol. 2019;1:8481375.

[4] Malfertheiner P, Megraud F, O'Morain CA, et al. Management of H. pylori infection-the Maastricht V/Florence consensus report. Gut. 2017;66:6–30.

[5] Sanches BS, Martins GM, Lima K, et al. Detection of Helicobacter pylori resistance to clarithromycin and fluoroquinolones in Brazil: a national survey. World J Gastroenterol. 2016;22:7587–94.

[6] Li Y, Lin R, Jin Y, et al. Genotyping Helicobacter pylori antibiotic resistance and virulence-associated genes in patients with gastric cancer in Wenzhou, China. Arab J Gastroenterol. 2021;22:267–71.

[7] Dore MP, Pes GM. What is new in Helicobacter pylori diagnosis. An overview. J Clin Med. 2021;10:2091.

[8] Kocazeybek B, Tokman HB. Prevalence of primary antimicrobial resistance of H. pylori in Turkey: a systematic review. Helicobacter. 2016;21:251–60.

[9] Cağdaş U, Otağ F, Tezcan S, et al. Detection of H. pylori and antimicrobial resistance in gastric biopsy specimens [in Turkish]. Mikrobiyol Bul. 2012;46:398–409.

[10] Caliskan R, Tokman HB, Erzin Y, et al. Antimicrobial resistance of H. pylori strains to five antibiotics, including levofloxacin, in Northwestern Turkey. Rev Soc Bras Med Trop. 2015;48:278–84.

[11] Güven B, Gülerman F, Kaçmaz B. H. pylori resistance to clarithromycin and fluoroquinoloness in a pediatric population in Turkey: a cross-sectional study. Helicobacter. 2019;24:e12581.

[12] Mégraud F. H. pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut. 2004;53:1374–84.

[13] Kocazeybek B, Sakli MK, Yuksel P, et al. Comparison of new and classical point mutations associated with clarithromycin resistance in H. pylori strains isolated from dyspeptic patients and their effects on phenotypic clarithromycin resistance. J Med Microbiol. 2019;68:566–73.

[14] Hu Y, Zhu Y, Lu NH. Primary antibiotic resistance of H. pylori in China. Dig Dis Sci. 2017;62:1146–54.

[15] Miftahussurur M, Shrestha PK, Subsomwong P, et al. Emerging H. pylori levofloxacin resistance and novel genetic mutation in Nepal. BMC Microbiol. 2016;16:256.

[16] Rajper S, Khan E, Ahmad Z, et al. Macrolide and fluoroquinolones resistance in H. pylori isolates: an experience at a tertiary care centre in Pakistan. J Pak Med Assoc. 2012;62:1140–44.

[17] Navarro-Jarabo JM, Fernández-Sánchez F, Fernández-Moreno N, et al. Prevalence of primary resistance of H. pylori to clarithromycin and levofloxacin in southern Spain. Digestion. 2015;92:78–82.