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 (86%, 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, levofloxacin

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 causal 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%.[1,4] 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.[11] 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.[8,10] In a recent study that used molecular methods, the rate of quinolone resistance was
reported to be 15% in the pediatrics population aged 2 to 18 years. 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

| Variables | Clarithromycin resistant (n = 50) | Levofoxacin resistant (n = 32) |
|-----------|----------------------------------|-------------------------------|
|           | % (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 (23) Ref | Ref | 26.7 (16) Ref | 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 | Ref | 20.9 (9) Ref | 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        | 16 (13.8)       | 100 (86.2)    | 116 (100) |

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. In the eradication of H pylori in Turkey, the antibiotic heteroresistance rate in a pediatric study in Ankara (50%), Izmir (48.2%), Bursa (41.9%), and Istanbul (41.9%) and indicate that this antibiotic has become unusable in our patients. In the eradication of H pylori in Turkey, the antibiotic heteroresistance rate in a pediatric study in 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. 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.

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 point mutation was A2143G (n = 11, 45.8%).[13] Also in the study, the high 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.[11] 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.[11,13]

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.

Statistical analyses, χ² test was used, and P values <.05 were considered statistically significant.

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) |
|               |             |                                 | gyr87MUT (N87K) | 4 (12.5) |
|               |             |                                 | gyr91MUT1 (D91N) | 9 (28.1) |
|               |             |                                 | gyr91MUT2 (D91G) | 11 (34.4) |
|               |             |                                 | gyr91MUT3 (D91Y) | 2 (6.3) |
|               |             |                                 | gyr91MUT2 (D91G) | 2 (6.3) |
|               |             |                                 | gyr87MUT (N87K) | 4 (12.5) |
|               |             |                                 | 23SMUT1 (A2146G) | 6 (37.5) |
|               |             |                                 | 23SMUT2 (A2146C) | 6 (37.5) |
|               |             |                                 | 23SMUT3 (A2147G) | 5 (31.2) |
|               |             |                                 | gyr91MUT1 (D91N) | 1 (6.2) |
|               |             |                                 | gyr91MUT2 (D91G) | 1 (6.2) |
|               |             |                                 | gyr91MUT3 (D91Y) | 1 (6.2) |
|               |             |                                 | gyr91MUT2 (D91G) | 1 (6.2) |
| Levofloxacin  | gyrA        | 32                              | 23SMUT1 (A2146G)| 2 (4) |
|               |             |                                 | 23SMUT2 (A2146C)| 0    |
|               |             |                                 | 23SMUT3 (A2147G)| 48 (96) |
|               |             |                                 | gyr87MUT (N87K) | 4 (12.5) |
|               |             |                                 | gyr91MUT1 (D91N) | 9 (28.1) |
|               |             |                                 | gyr91MUT2 (D91G) | 11 (34.4) |
|               |             |                                 | gyr91MUT3 (D91Y) | 2 (6.3) |
|               |             |                                 | gyr91MUT2 (D91G) | 2 (6.3) |
|               |             |                                 | gyr87MUT (N87K) | 4 (12.5) |
|               |             |                                 | 23SMUT1 (A2146G) | 6 (37.5) |
|               |             |                                 | 23SMUT2 (A2146C) | 6 (37.5) |
|               |             |                                 | 23SMUT3 (A2147G) | 5 (31.2) |
|               |             |                                 | gyr91MUT1 (D91N) | 1 (6.2) |
|               |             |                                 | gyr91MUT2 (D91G) | 1 (6.2) |
| Dual resistance | 23S rRNA    | 16                              | gyr91MUT2 (D91G) | 1 (6.2) |
|               | plus gyrA   |                                 | gyr91MUT3 (D91Y) | 1 (6.2) |
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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 Istamb. 2019;7:87–94.
[2] Vilasichone 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, Rüttergerdt N, Bohne W, et al. H. pylori mutations conferring resistance to fluoroquinolones 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] Çağdaş 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, Guílerman E, Kaçmaz B. H. pylori resistance to clarithromycin and fluoroquinolones 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.