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Research Article

Detection of Antibiotic Resistance by Phenotypic and Genotypic Methods in Helicobacter Pylori and its Association with Genotypes

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Abstract

Objectives: This study aimed to determine the prevalence of Helicobacter pylori infection in patients with dyspeptic symp-toms, to evaluate antibiotic resistance rates using both phenotypic and genotypic methods, and to investigate the association between resistance and the presence of cagA and vacA virulence genotypes.

Methods: A total of 103 consecutive patients (64 female, 39 male; mean age 47.7 +/- 15.9 years) who underwent upper gastrointestinal endoscopy for dyspepsia were included in this cross-sectional study. Biopsy samples were obtai-ned from both the antrum and corpus of each patient. *H. pylori* culture was performed on selective media under microaerophilic conditions. Antibiotic susceptibility testing for amoxicillin, clarithromycin, tetracycline, and le-vofloxacin was conducted using the E-test method. DNA extracted from biopsy samples was amplified by polymerase chain reaction (PCR) using specific primers targeting the glmM, cagA, and vacA genes. Clarith-romycin resistance was confirmed by PCR-restriction fragment length polymorphism (PCR-RFLP) targeting the 23S rRNA gene. Levofloxacin resistance was confirmed by DNA sequence analysis of the gyrA gene. Histopat-hological examination was performed using Giemsa staining.

Results: *H. pylori* positivity was detected in 76 of 103 patients (73.8%) by glmM PCR, whereas culture positivity was observed in only 58 patients (56.3%), indicating significantly higher sensitivity of PCR ($p < 0.001$). Among the 58 culture-positive isolates, E-test resistance rates were as follows: amoxicillin 1.7% (1/58), clarithromycin 10.3% (6/58), tetracycline 0% (0/58), and levofloxacin 6.9% (4/58). No statistically significant association was found between antibiotic resistance and patient gender or endoscopic findings ($p > 0.05$). Among the 76 PCR-positive samples, the cagA gene was positive in 46 (60.5%) and the vacA gene was positive in 73 (96.0%). The ca-gA+/vacA+ (Type I) genotype was present in 46 patients (60.5%). No significant association was detected between antibiotic resistance and the presence of cagA or vacA genotypes ($p > 0.05$). Molecular analysis revea-led that among clarithromycin-resistant isolates, the A2143G mutation was present in 71.4% (5/7) and the A2142G mutation in 28.6% (2/7). All four levofloxacin-resistant isolates harbored gyrA point mutations: Asp-91 in three isolates (75%) and Asn-87 in one isolate (25%).

Conclusion: The clarithromycin resistance rate (10.3%) in the Kahramanmaraş region remains within the range reported in European countries and below the 15% threshold. However, levofloxacin resistance (6.9%) is emerging and requires careful monitoring. Amoxicillin and tetracycline resistance remain low. No association was found between cagA or vacA genotypes and antibiotic resistance. PCR-based methods demonstrated superior sensiti-vity compared to culture for *H. pylori* detection. Periodic regional surveillance of antibiotic resistance patterns is essential to guide appropriate empiric treatment and preserve the efficacy of *H. pylori* eradication regimens.

Keywords: *Helicobacter pylori*, antibiotic resistance, clarithromycin, levofloxacin, cagA, vacA

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More than half of the global population carries Helicobacter pylori in their stomachs. This Gram-negative, spiral-shaped, microaerophilic organism colonizes the gastric lining and serves as a primary cause of several upper gastrointestinal disorders, including persistent gastritis, peptic ulcers, gastric cancer, and MALT lymphoma.[1] Prevalence rates vary considerably between nations: developing countries report figures between 60% and 85%, whereas developed nations have seen declines to 10–30% owing to better sanitation and effective treatment protocols.[2] Turkish studies indicate an average infection rate of approximately 85%.[3]

Here is the edited text. As requested, all edits strictly address grammar, punctuation, and typographical consistency, with zero use of LaTeX formatting.

Standard first-line therapy for H. pylori combines a proton pump inhibitor with two antibiotics—amoxicillin and clarithromycin. Nevertheless, treatment fails in 15–40% of cases, largely because of emerging primary and acquired resistance, especially toward clarithromycin and levofloxacin.[4,5] According to the Maastricht III consensus, clarithromycin-based regimens should be administered cautiously in areas where resistance rates are elevated.[6]

Geographic differences in resistance patterns are substantial. European studies report clarithromycin resistance between 2% and 15%, while Turkish data show figures exceeding 27%.[7,8] Worldwide, resistance to amoxicillin and tetracycline remains uncommon (<1% and <0.5–9%, respectively).[7] Fluoroquinolone resistance, particularly to levofloxacin, is an increasing global concern.[9]

Among the bacterial factors influencing disease severity, cagA (cytotoxin-associated gene A) and vacA (vacuolating cytotoxin A) are the most extensively studied. Strains carrying cagA (designated Type I) have been linked to more serious clinical manifestations, including peptic ulcers and gastric malignancy.[10,11] Whether these virulence determinants correlate with antibiotic susceptibility, however, remains poorly understood.

The present investigation sought to assess H. pylori infection frequency, establish phenotypic and genotypic resistance profiles for amoxicillin, clarithromycin, tetracycline, and levofloxacin, and explore potential links between resistance and cagA/vacA genotypes among dyspeptic patients from the Kahramanmaraş province of Türkiye.

Methods

Study Population

This cross-sectional investigation enrolled 103 consecutive dyspeptic patients (64 women, 39 men; mean age 47.7±15.9 years) who underwent upper gastrointestinal endoscopy at Kahramanmaraş Sütçü İmam University Faculty of Medicine's Gastroenterology Clinic between January and June 2013. Individuals were excluded if they had previously received H. pylori eradication, used antibiotics or PPIs within 12 weeks before enrollment, were pregnant or lactating, had a history of gastroduodenal surgery, or reported allergies to the study antibiotics.

This study was approved by the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine with decision number 2013/01-7 on January 29, 2013, and all participants provided written informed consent.

Endoscopy and Biopsy Collection

Upper gastrointestinal endoscopy was performed using a fiberoptic endoscope. Five biopsy samples were obtained from each patient: two from the antrum and two from the corpus (one each for culture and PCR) and one for histopathological examination. Biopsy samples for culture were placed in Brain Heart Infusion Broth containing 3% glycerol and transported to the microbiology laboratory within 2 hours.

Culture and Identification

After mechanical homogenization, biopsy materials were plated onto modified Columbia Agar enriched with 7% horse blood, Helicobacter selective supplement (Dent, Oxoid, Hampshire, England), and Vitox supplement (Oxoid). Plates were kept under microaerophilic conditions (5% O2, 10% CO2, 85% N2) at 37°C for 4–7 days. Colonies exhibiting suspicious growth were confirmed as H. pylori through Gram staining and positive oxidase, catalase, and urease reactions.[12]

Antibiotic Susceptibility Testing (E-test)

Susceptibility testing employed the E-test method (bioMérieux, France). A bacterial suspension matching McFarland 3 turbidity was spread onto Mueller-Hinton Agar supplemented with 7% horse blood. E-test strips for amoxicillin, clarithromycin, tetracycline, and levofloxacin were placed on the inoculated plates. Following microaerophilic incubation at 37°C for 3–5 days, MIC values were determined at the point where the inhibition ellipse intersected the strip. Resistance interpretation followed the British Society for Antimicrobial Chemotherapy (BSAC) 2013 criteria.[13]

DNA Extraction and PCR

The QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from biopsy specimens according to the manufacturer's instructions. H. pylori presence was confirmed by amplifying the glmM gene (294 bp).[14] Detection of cagA (349 bp) and vacA (259/286 bp) was per-

formed using specific primer sets. PCR products underwent electrophoresis on 2% agarose gels and were visualized with ethidium bromide staining.

Molecular Detection of Clarithromycin Resistance (PCR-RFLP)

A 135 bp segment of the 23S rRNA gene was amplified. The resulting products were digested with *MboII* (to detect the A2142G mutation) and *Eco3II* (to detect the A2143G mutation) restriction enzymes (Fermentas, USA). Digestion patterns were resolved on 3.5% agarose gels.[15]

Molecular Detection of Levofloxacin Resistance (DNA Sequencing)

Amplification of a 428 bp fragment of the *gyrA* gene was carried out. Purified PCR products were sequenced using the Sanger dideoxy chain-termination method on an automated sequencer. Obtained sequences were aligned with the reference *H. pylori* 26695 strain.[16]

Histopathological Examination

Biopsy samples fixed in 10% formalin were paraffin-embedded, sectioned, and stained with Giemsa for microscopic evaluation of *H. pylori* and gastritis assessment.

Statistical Analysis

SPSS version 21.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. Continuous variables are presented as mean ± standard deviation, while categorical variables are shown as numbers and percentages. Group comparisons employed Student's t-test for continuous data and the chi-square test for categorical data. Statistical significance was defined as $p<0.05$.

Artificial Intelligence Statement

During the preparation of this work, the authors used DeepSeek (a Large Language Model) for language editing, grammar correction, and formatting assistance. All AI-generated suggestions were reviewed, fact-checked, and approved by the corresponding author. No AI was used for data collection, statistical analysis, interpretation of results, or conclusion generation. The authors take full responsibility for the final content of the manuscript.

Results

Patient Demographics and H. pylori Positivity

The study group comprised 103 individuals, of whom 64 (62.1%) were female and 39 (37.9%) were male, with an average age of 47.7±15.9 years. *H. pylori* was detected in 56.3% (58/103) of patients by culture and in 73.8% (76/103) by *glmM* PCR, demonstrating that PCR is significantly more sensitive ($p<0.001$).

Endoscopic and Histopathological Findings

Among the 103 patients, 40 (38.9%) presented with gastric or duodenal ulcers or mucosal lesions (Group A), whereas 63 (61.1%) showed normal mucosa (Group B). *H. pylori* positivity rates were 75.0% (30/40) in Group A and 73.1% (46/63) in Group B, with no statistically significant difference ($p>0.05$). Histopathological examination identified *H. pylori* in 75 patients (72.8%).

Antibiotic Resistance Rates (Phenotypic)

Table 1 summarizes E-test results for the 58 culture-positive isolates. Resistance rates were as follows: amoxicillin 1.7% (1/58), clarithromycin 10.3% (6/58), tetracycline 0% (0/58), and levofloxacin 6.9% (4/58). No significant associations emerged between resistance and either patient sex or endoscopic group ($p>0.05$ for all comparisons).

Genotype Distribution

Among the 76 PCR-positive samples, *cagA* was positive in 46 (60.5%), and *vacA* was positive in 73 (96.0%). The combined *cagA*+/*vacA*+ (Type I) genotype occurred in 46 patients (60.5%). *cagA* positivity was 70.0% (21/30) in Group A versus 54.3% (25/46) in Group B ($p>0.05$) (Table 2).

Association Between Genotypes and Antibiotic Resistance

No statistically significant relationship was observed between antibiotic resistance and the presence of either *cagA* or *vacA* ($p>0.05$ for all comparisons) (Table 3).

Table 1. Antibiotic resistance rates determined by E-test (n=58)

Antibiotic	Susceptible n (%)	Resistant n (%)	Resistance rate (%)
Amoxicillin	57 (98.3)	1 (1.7)	1.7
Clarithromycin	52 (89.7)	6 (10.3)	10.3
Tetracycline	58 (100)	0 (0)	0
Levofloxacin	54 (93.1)	4 (6.9)	6.9

Table 2. Distribution of *cagA* and *vacA* genotypes according to endoscopic findings

Endoscopic Group	*cagA* Positive n (%)	*vacA* Positive n (%)
Group A (n=30)	21 (70.0)	29 (96.6)
Group B (n=46)	25 (54.3)	44 (95.6)
p	>0.05	>0.05

Group A: Patients with gastroduodenal ulcer/mucosal lesions; Group B: Patients with normal mucosa.

Table 3. Antibiotic resistance according to *cagA* and *vacA* status

Virulence Factor	Amoxicillin R	Clarithromycin R	Levofloxacin R
cagA Positive (n=46)	1 (2.2%)	4 (8.7%)	3 (6.5%)
cagA Negative (n=30)	0 (0%)	2 (6.7%)	1 (3.3%)
vacA Positive (n=73)	1 (1.4%)	6 (8.2%)	4 (5.5%)
vacA Negative (n=3)	0 (0%)	0 (0%)	0 (0%)

R: resistant; no statistically significant differences were observed (p>0.05 for all)

Table 4. Molecular characterization of antibiotic resistance mutations

Antibiotic	Mutation	n	Frequency (%)
Clarithromycin (n=7)	A2143G	5	71.4
	A2142G	2	28.6
Levofloxacin (n=4)	*gyrA* Asp-91	3	75.0
	gyrA Asn-87	1	25.0

Molecular Resistance Mechanisms

Of the seven clarithromycin-resistant isolates (six phenotypic plus one molecular), PCR-RFLP identified the A2143G mutation in five (71.4%) and the A2142G mutation in two (28.6%).[15] All four levofloxacin-resistant isolates carried *gyrA* point mutations: Asp-91 in three isolates (75%) and Asn-87 in one isolate (25%)[16] (Table 4).

Discussion

The present work offers a detailed examination of *H. pylori* infection frequency, antibiotic resistance patterns, and virulence genotype distribution within the Kahramanmaraş region of Turkey. The 73.8% positivity rate detected by PCR aligns with the elevated prevalence typical of developing countries and matches earlier Turkish investigations reporting figures between 60% and 85%.[17] PCR proved markedly more sensitive than culture (73.8% versus 56.3%, p<0.001), underscoring the advantage of molecular techniques for *H. pylori* detection, especially when bacterial viability may be compromised during specimen transport.[18]

The 10.3% clarithromycin resistance rate observed here falls within the 2–15% range documented across Europe and remains below the 15% threshold. This figure is substantially lower than the 27–56% reported in some recent Turkish studies.[19] Such discrepancies likely stem from regional differences in antibiotic utilization and highlight the necessity for local resistance monitoring. The predominance of the A2143G mutation (71.4%) among clarithromycin-resistant isolates concurs with earlier findings that this alteration is the most frequent cause of high-level macrolide resistance.[15]

The 6.9% levofloxacin resistance rate is a matter of concern, given that fluoroquinolone resistance has been rising worldwide due to extensive use of these agents across various infections.[9] Although this rate is lower than the 16.8–23% reported in certain European countries[20] and the 21.5% from Korea,[21] any detectable levofloxacin resistance carries clinical importance, as fluoroquinolones are increasingly employed in second-line and salvage therapies. The identification of *gyrA* mutations (Asp-91 and Asn-87) in all resistant isolates confirms that these point mutations constitute the primary mechanism underlying fluoroquinolone resistance in *H. Pylori*.[16]

Amoxicillin (1.7%) and tetracycline (0%) resistance remained infrequent, consistent with global data.[7] These agents continue to hold value as components of *H. pylori* eradication regimens, particularly within quadruple therapy protocols.

The 60.5% *cagA* positivity rate observed falls within the 50–70% range typical of Western populations.[10,11] While *cagA*-positive strains are generally associated with more aggressive clinical courses, the lack of a statistically significant relationship between *cagA* status and endoscopic findings (70.0% in Group A versus 54.3% in Group B, p>0.05) suggests that host factors and other bacterial determinants also contribute significantly to disease outcomes.[22]

Importantly, no association emerged between *cagA* or *vacA* genotypes and antibiotic resistance. This finding indicates that a strain's virulence potential does not predict its antimicrobial susceptibility profile, nor does resistance development appear to be influenced by the presence of these virulence genes.

Study Limitations

Several limitations warrant acknowledgment. First, the sample size was relatively modest, and the single-center design may limit generalizability. Second, metronidazole resistance—which is highly prevalent in many regions—was not assessed. Third, only the signal region of *vacA* was examined; the mid-region, which also modulates toxin activity, was not analyzed.

Conclusion

In the Kahramanmaraş region, the clarithromycin resistance rate (10.3%) remains within acceptable limits, whereas levofloxacin resistance (6.9%) is an emerging concern that warrants vigilant monitoring. Amoxicillin and tetracycline resistance stay low. No relationship exists between *cagA* or *vacA* genotypes and antibiotic resistance. PCR-based methods surpass culture in sensitivity for *H. pylori* detection. Regular regional surveillance of antibiotic resistance profiles is crucial to inform appropriate empiric therapy and preserve the effectiveness of *H. pylori* eradication regimens.

Disclosures

Ethical Committee Approval: This study was approved by the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine with decision number 2013/01-7 on January 29, 2013.

Informed Consent: All participants provided written informed consent.

Conflict of Interest: The authors declare no conflict of interest.

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