



A comprehensive study of *Helicobacter pylori* infection: molecular analysis, antibacterial susceptibility, and histopathological examination

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Abstract *Helicobacter pylori* is a pathogen associated with gastroduodenal diseases. This study aimed; (i) to investigate *H. pylori* presence by invasive tests in adult dyspeptic patients, (ii) to determine antibiotic susceptibility and genotypic characteristics of the *H. pylori* isolates, and (iii) to investigate the relationship between the *H. pylori* genotypes and the histopathological findings. In this cross-sectional study, gastric biopsy samples from 208 adult dyspeptic patients were used for culture, tissue Polymerase Chain Reaction (PCR), and histopathological analysis. Antibiotic susceptibility of the *H. pylori* isolates was analyzed by gradient method. Analysis of the virulence genes was performed by monoplex PCR. Genetic profiles (from A to H) were created based on the virulence genes presence. Enterobacterial Repetitive Intergenic

Consensus-PCR (ERIC-PCR) was used for the genotyping of the *H. pylori* isolates. The mean age of the patients was 46 (± 15) years and 128 (61.5%) of them were female. *H. pylori* positivity was detected by culture, tissue PCR and histopathological examination in 59 (28.4%), 114 (54.8%) and 81 (38.9%) patients, respectively. The overall prevalence of *H. pylori* was found to be 63% (131/208). All *H. pylori* isolates were susceptible to tetracycline and amoxicillin. The resistance rates for metronidazole, clarithromycin, levofloxacin, and rifampicin were 67.2%, 27.9%, 34.4% and 13.11%, respectively. Multi drug resistance (MDR) was detected at the rate of 45.9% (28/61). While the most common virulence gene was *cagA* (93.44%), the least common was *vacAm1* (23%). The predominant genetic profile was profile

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A (47.5%). ERIC-PCR results revealed a total of 26 different patterns. A high prevalence of *H. pylori* was detected in adult dyspeptic patients as in developing countries. It was observed significant genotypic heterogeneity and virulence gene diversity within the isolates. A considerable resistance rate detected against antibiotics such as clarithromycin, metronidazole, and levofloxacin, which are frequently used in the eradication of *H. pylori*, should be taken into consideration when creating regional empirical treatment regimens.

Keywords Antibiotic resistance · Dyspepsia · Gastritis · Genotyping · *Helicobacter pylori* · Virulence gene

Introduction

Helicobacter pylori, which has a Gram-negative helical-spiral morphology, is a gastric pathogen. It is known that more than half of the people in the world are infected with *H. pylori*.

While the prevalence of the causative agent is below 20% in developed countries, this rate can reach 90% in developing countries (Hooi et al. 2017). The population-based prevalence of *H. pylori* in Türkiye was found to be 82.5% in a study conducted with the urea breath test (Ozaydin et al. 2013).

Helicobacter pylori is associated with chronic gastritis, peptic ulcer, gastric cancer and gastric malignancy (Malfertheiner et al. 2017). The clinical reflection of the infection differs according to the host and environmental determinants, as well as the virulence factors of the agent. Nutritional habits, gender, age, alcohol uses and smoking, and additional diseases are considered as host-related factors. The clinical course of the infection can also be changed by various virulence factors involved in the pathogenesis (Gonzalez Vazquez et al. 2016; Kishk et al. 2021). The most important of these factors are *cagA* and *s1*, *s2*, *m1* and *m2* alleles of the *vacA* gene (Cover and Blanke 2005; Higashi et al. 2002; Roesler et al. 2014). It is thought that the *cagA* and *vacA* genes of *H. pylori*, which show diversity in distribution due to population and geographical differences, also affect the prognosis of the infection and its response to the treatment (Akar et al. 2022; Broutet et al. 2001; Oliveira et al. 2021; Parsonnet et al. 1997; Sugimoto and Yamaoka 2009). It was also emphasized that there may be a

close relationship between the presence of virulence genes and antibiotic resistance (Boyanova et al. 2010; Brennan et al. 2018; Elviss et al. 2004).

In the treatment of *H. pylori* infection, double or triple empirical antibiotic combination is used in addition to high-dose proton pump inhibitor (PPI) (O'Connor et al. 2017; Sezgin et al. 2019). However, increasing rates of resistance to antibiotics such as metronidazole, clarithromycin, levofloxacin and rifampicin adversely affect the success of treatment. For this reason, it is best to know the current antibiotic susceptibility profiles of that region in order to develop the ideal regional treatment regimen (Arslan et al. 2017).

In this study it was aimed; (i) to investigate *H. pylori* positivity by invasive methods in adult dyspeptic patients, and (ii) to determine the antibiotic susceptibility and genotypic characteristics of the obtained isolates. In addition, the existence of a relationship among *H. pylori* positivity, genotypes and histopathological findings was also investigated.

Materials and methods

Patients and ethical approvals

In this study, gastric biopsy specimens obtained from 208 adult patients who admitted to the General Surgery Clinic of Kafkas University Faculty of Medicine between December 2019 and March 2020 with dyspeptic complaints and underwent upper gastrointestinal endoscopy were used. Demographic, clinical, endoscopic, and histopathological findings of the patients were recorded. Patients aged 18 years and older with dyspeptic complaints were included in the study. Patients with a history of gastrectomy, pregnant women, patients who used antibiotics in the last 6 weeks, patients who used proton pump inhibitors in the last 4 weeks, patients with bleeding diathesis and who had previously received eradication therapy for *H. pylori* were excluded from the study. This study was conducted in accordance with the criteria in the Declaration of Helsinki, and written informed consent was obtained from all patients included in the study. Ethics committee approval of the study was obtained from the Ethics Committee of the Faculty of Medicine, Kafkas University (Kars, Türkiye) (2019/26).

Gastric biopsy samples

During the upper gastrointestinal endoscopic examination, one antrum and one corpus biopsy samples were taken with forceps and put into 10% formol for histopathological examination. In addition, two biopsy samples (one corpus and one antrum) were taken and transferred to cryotubes containing 500 µl Brain Heart Infusion Broth (BHI Broth, CM1135B, Thermo Fisher Scientific, USA) for cultural examination and molecular analysis (Polymerase Chain Reaction, PCR) and kept at -80°C until testing.

Cultural analysis

The biopsy samples in the cryotube were crushed with a sterile glass baguette for 5–10 min, then 20 µl of homogenate was directly plated onto Columbia Blood agar (CM0331B, Thermo Fisher Sci., USA) plates supplemented with 7% horse blood and Dent supplement (SR0147E, Thermo Fisher Sci., USA). The inoculated media were incubated at 37°C for 7–10 days in a humid microaerobic environment (Anaerocult @C , 2.5 L, 132,383, Merck, Germany). Gram staining, morphology, motility, catalase, oxidase, and urease activities of smooth (S) colonies grown after incubation were determined and evaluated phenotypically in terms of *H. pylori* (Otth et al. 2011).

Molecular analysis

DNA extraction

DNA extraction from both *H. pylori* isolates obtained from cultural examination and gastric biopsy samples was performed using the commercial kit (QIAamp DNA Mini Kit, 56,304, Qiagen, DE) according to the manufacturer's instructions.

Helicobacter pylori specific PCR

Molecular confirmation of the isolates identified as *H. pylori* by the phenotypic method was performed by PCR, which amplifies the phosphoglucosamine mutase gene (*glmM*) specific for *H. pylori* (Lu et al. 1999). HpF (5'-AAGCTTTTAGGGGTGTTAGGG GTTT-3') and HpR (5'-AAGCTTACTTTCTAACAC TAACGC-3') primers were used in the PCR. The

same PCR method was used to detect the presence of *H. pylori* in gastric biopsy samples. Amplified products were analyzed by 1.5% agarose gel electrophoresis (Biomax, Agarose, Lot no. 124543PR, from Prona, European Economic Community) and visualized under a UV transilluminator (G:BOX Chemi XRQ; Syngene, Cambridge, UK). Bands with 294-bp in size, were considered as positive for *H. pylori*.

Antibiotic susceptibility testing

Gradient method was used to determine the antibiotic susceptibility of the *H. pylori* isolates. Clarithromycin (CH), amoxicillin (AC), tetracycline (TC), metronidazole (MZ), levofloxacin (LE) and rifampicin (RI) (Biomerieux, France) E-test strips were used. Bacterial inoculum was prepared according to McFarland No. 3 standard (9×10^8 cfu/ml) with Mueller–Hinton (MH) Broth (CM0405B, Thermo Fisher Sci., USA). One hundred µl of the bacterial suspension was inoculated on the MH agar (CM0337B, Thermo Fisher Sci., USA) plates supplemented with 7% horse blood and spread with a L-shaped spreader and E-test strips were placed. The media were incubated in a microaerobic condition at 37°C for 3 days. Sensitivity/resistance interpretation of the isolates was performed according to EUCAST guidelines (EUCAST 2022). Resistance to two or more antibiotics was defined as multidrug resistant (MDR).

Histopathological analysis

Gastric biopsy samples were stained with hematoxylin–eosin (Fallone et al. 1997) and evaluated by an expert pathologist according to the Sydney classification for the degree of inflammation, gastric atrophy, intestinal metaplasia, and dysplasia (Manxhuka-Kerliu et al. 2009). The samples were also stained with Giemsa and evaluated for the presence of *H. pylori* (Rotimi et al. 2000).

Evaluation of the *Helicobacter pylori* presence

Three invasive tests (culture, histopathology and tissue PCR) were used for the evaluation of *H. pylori* positivity. Thus, the positivity of any of these tests was accepted as the overall prevalence, while the positivity of all three tests at the same time was defined as intersect prevalence.

Virulence genes analysis

The virulence genes of *cagA*, *vacAm1*, *vacAm2*, *vacAs1* and *vacAs2* of the *H. pylori* isolates were analyzed by PCR. Primer *cagA*-F (5'-GATAACAGGCAAGCTTTTGAGG-3') and *cagA*-R (5'-CTGCAAAGATTGTTTGGCAGA-3') (Tummuru et al. 1993) for the amplification of the *cagA* gene; VAG-F (5'-CAATCTGTCCAATCAAGCGAG-3') and VAG-R (5'-GCGTCAAAATAATTCCAAGG-3') (Atherton et al. 1995) for the *vacAm1/m2* genes and VA1-F (5'-ATGGAAATACAACAAACACAC-3') and VA1-R (5'-CTGCTTGAATGCGCCAAAC-3') (Houf et al. 2002) for the *vacAs1/s2* genes were used. Each virulence gene analysis was performed separately by PCR. Amplified products were analyzed by 1.5% agarose gel electrophoresis and products with sizes of 349 bp, 567 bp, 642 bp, 259 bp and 286 bp were evaluated as positive for *cagA*, *vacAm1*, *vacAm2*, *vacAs1* and *vacAs2*, respectively (Fig. 1). Genetic profiles were created according to the different virulence genes existence.

Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR)

Genotyping of the *H. pylori* isolates obtained by cultural analysis was performed by ERIC-PCR according to the method reported by Versalovic et al. (1991). This is a genotyping method that discriminates bacterial strains by amplifying the regions between ERIC sequences called 127 bp in length repetitive imperfect palindromes found in as multiple copies on bacterial genomes (Wilson and Sharp 2006). For this purpose,

primers 1R (5'-ATGTAAGCTCCTGGGGATTACAC-3') and 2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') were used. Electrophoresis of the amplified products was performed on a 2% agarose gel. The banding pattern from ERIC-PCR was analyzed with BioNumerics Software version 7.6 (AppliedMaths, Ghent, Belgium). The similarities between strains were calculated using the Dice coefficient. The dendrogram was constructed by means of the Unweighted Pair Group Method with Arithmetic Average (UPGMA) clustering algorithm. Similarity index > 80% was used to assign clusters (Fendri et al. 2013; Saticioglu et al. 2018; Wijetunge et al. 2012).

Statistical analysis

SPSS 21.0 (Statistical Packages for Social Sciences; SPSS Inc., Chicago, Illinois, USA) and MedCalc-20.015 program were used for statistical analysis of the data obtained in the study. Data that could be measured and provided the parametric condition were given as mean $\pm$ standard deviation. Categorical variables were shown as numbers and percentages. Chi-square test was used to compare groups with categorical variables. $P < 0.05$ was considered significant in all statistical evaluations.

Results

Clinical data and diagnostic tests results

The mean age of the patients was 46 ± 15 (range: 19–86) years, and 128 (61.5%) were female. Gastritis,

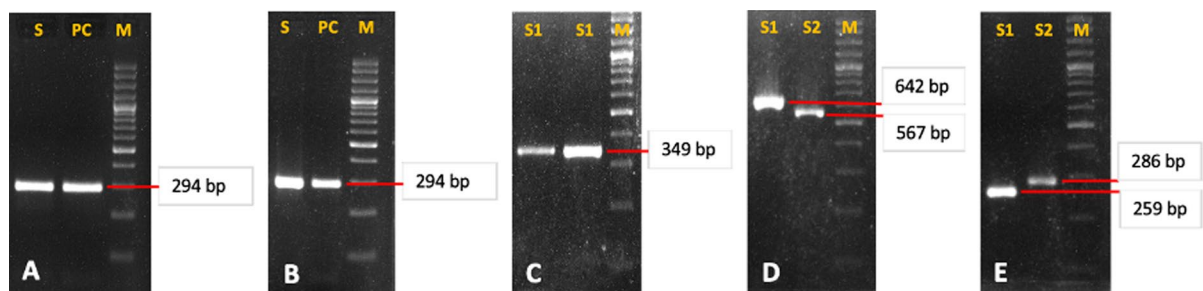


Fig. 1 PCR findings of identification and genotyping of *H. pylori* isolates. **A** Image of tissue PCR (294 bp), **B** Species-specific PCR image of culture positive samples (294 bp), **C–E** Virulence gene PCR findings of *H. pylori* isolates (*cagA* 349 bp, *vacAm1* 567 bp and *vacAm2* 642 bp, *vacAs1* 259 bp

and *vacAs2* 286 bp, respectively). S, S1, S2: *H. pylori* field isolates, PC: Positive control (*H. pylori* NCTC 11637), M: DNA ladder (GeneRuler 100 bp plus DNA ladder, SM 0321, Thermo Fisher Sci)

reflux esophagitis, and peptic ulcer were detected in 124 (59.6%), 35 (16.8%) and 47 (22.6%) patients, respectively, in the endoscopic examination (Table 1).

A total of 59 (28.4%) patients were found to be positive for *H. pylori* by the cultural method. Two different *H. pylori* colonies were detected in cultural analyses of two biopsy samples (#16 and #179). Thus, 61 (29.3%) *H. pylori* isolates were obtained

from 208 gastric biopsy samples (Fig. 2). The youngest and oldest patients with *H. pylori* isolated were 20 and 75 years old. Tissue PCR revealed 114 (54.8%) patients positive for the presence of *H. pylori*. As a result of histopathological analysis, *H. pylori* positivity was detected in 81 (38.9%) patients. The intersect prevalence of *H. pylori* was 17.8% (37/208), while the overall prevalence was 63% (131/208) (Table 1).

Antimicrobial testing results

While all of 61 *H. pylori* isolates were susceptible to AC and TC. The *H. pylori* isolates were found to be resistant to MZ, LE, CH, and RI at the rate of 67.2% (41/61), 34.4% (21/61), 27.9% (17/61), and 13.1% (8/61), respectively. MZ resistance was found at the rate of 67.2% (41/61) among the isolates (Fig. 2). The Minimum Inhibitory Concentration (MIC) distributions of the *H. pylori* isolates according to the EUCAST criteria were given in Table 2.

While 10 (16.4%) *H. pylori* isolates were not resistant to any antibiotic (no drug resistance-NDR), single drug resistance (SDR) and MDR were detected in 23 (37.7%) and 28 (45.9%) isolates, respectively. Within the MDR profiles, 36.1% (22 isolates) of the isolates exhibited dual (DDR), 6.6% (4 isolates) triple (TDR) and 3.3% (2 isolates) quadruple antibiotic resistance (QDR) (Fig. 3).

Histopathological analysis findings

It couldn't be reached to histopathological analyses results of seven patients. Therefore, the results belong to 201 patients were evaluated. Chronic gastritis was detected in a total of 179 (89.1%) patients by histopathological analysis. Adenocarcinoma was observed in two cases (4.3%) of 47 patients with peptic ulcer in the endoscopic evaluation. While inflammation

Table 1 Demographic and endoscopic data of the patients and test results used in the detection of *H. pylori* (n = 208)

Variables	Value
<i>Demographic findings</i>	
Age ($\pm$ SD), year	46 ($\pm$ 15) (range: 19–86)
Gender, n (%)	
Male	80 (38.5)
Female	128 (61.5)
<i>Endoscopic findings</i>	
Gastritis, n (%)	124 (59.6)
Reflux esophagitis, n (%)	35 (16.8)
Peptic ulcer, n (%)	47 (22.6)
<i>Diagnostic tests findings</i>	
Hp positivity by, n (%)	
Culture	59 (28.4)*
Tissue PCR	114 (54.8)
Histopathology	81 (38.9)**
Hp prevalence estimates, n (%)	
Prevalence intersect	37 (17.8)
Overall prevalence	131 (63)

SD Standard deviation, *Hp Helicobacter pylori*

*Four *H. pylori* isolates were simultaneously obtained from two gastric biopsy samples

**Three culture-positive gastric biopsy specimens have no histopathological analysis report

Fig. 2 Culture and antibiotic susceptibility test findings of *H. pylori* positivity. **A** 7–10 days culture results of *H. pylori* on Columbia Blood agar supplemented with the Dent. **B** and **C** E-test antibiotic susceptibility test results of *H. pylori* isolates in MH media

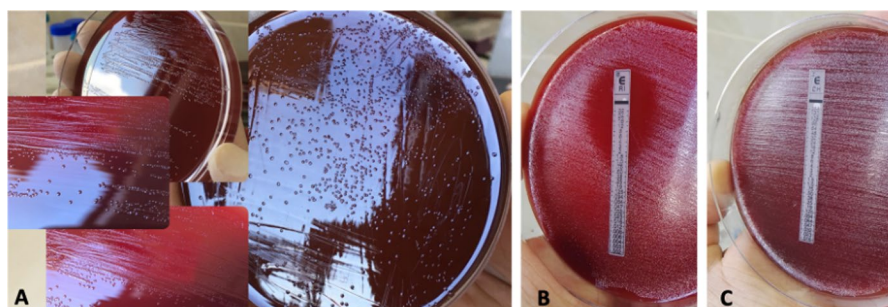


Table 2 MIC distributions of the *H. pylori* isolates (n = 61) according to EUCAST criteria

Antibiotics	MIC range		The number of the <i>H. pylori</i> isolates with an MIC of:																																							
	S _{IV}	R _{IV}	0.002	0.003	0.004	0.006	0.008	0.012	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	6	8	12	16	24	32	48	64	96	128	192	256					
AC	0.125	0.125								61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
CH	0.25	0.5								0	21	1	17	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0	0	0	0	12			
LE	1	1	0	0	0	0	0	0	0	12	2	0	3	0	0	1	1	0	0	21	0	0	0	0	0	0	0	1	0	2	0	18										
MZ	8	8								0	0	11	0	1	1	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	4	0	1	0	33				
RI	1	1	0	0	0	0	0	0	0	0	0	0	0	13	2	11	10	7	10	0	1	3	1	0	0	0	0	0	0	3												
TC	1	1								6	2	1	2	5	28	10	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			

Orange shades indicate the resistant isolates for each antibiotic. Grey shades are out of the E-test strip grades

MIC minimum inhibitory concentration, S sensitive, R resistant, AC Amoxicillin 0.016–256, TC Tetracycline 0.016–256, MZ Metronidazole 0.016–256, CH Clarithromycin 0.016–256, LE Levofloxacin 0.002–32, RI Rifampicin 0.002–32

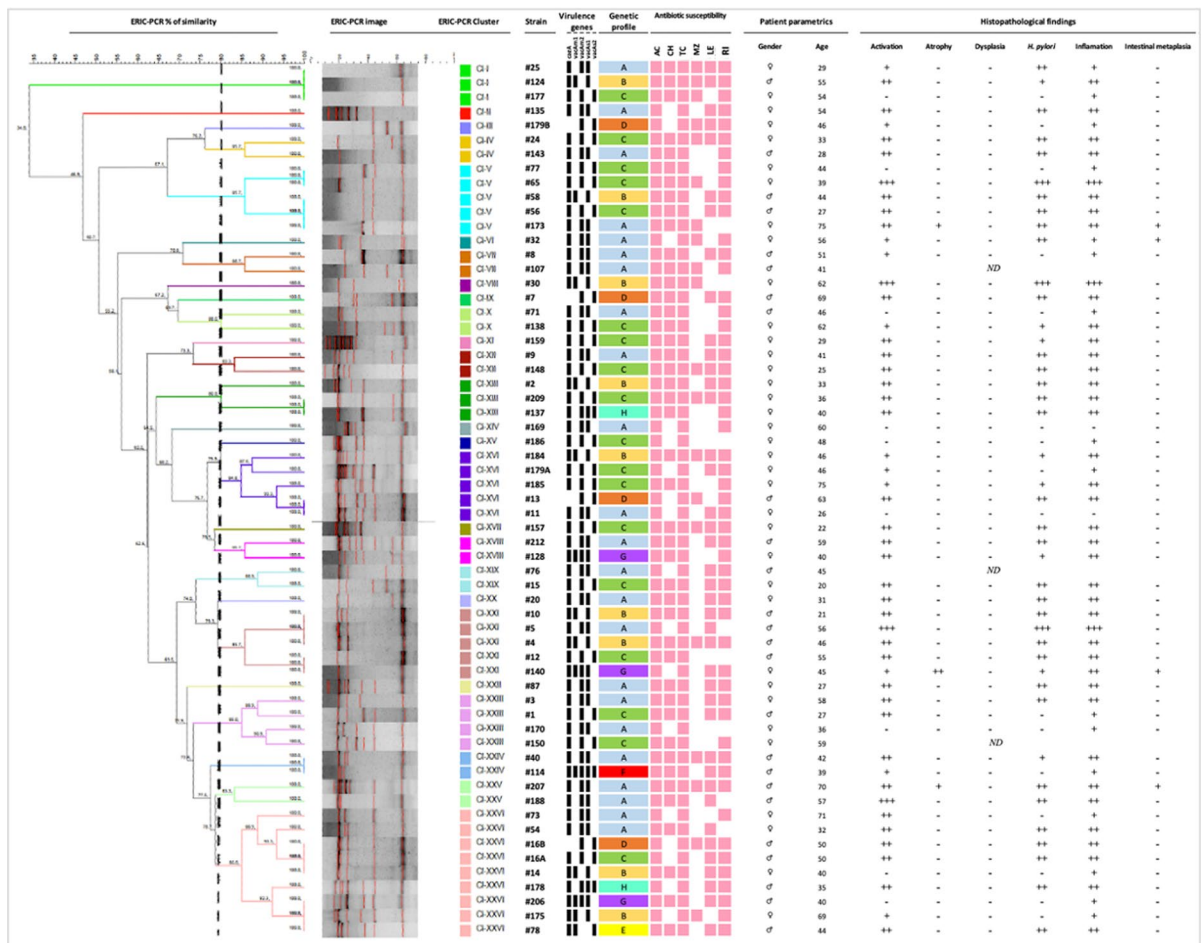


Fig. 3 Molecular characteristics and antibacterial susceptibility of the *H. pylori* isolates and demographical data and histopathological findings of the patients. ND: Not determined, —:

Negative, +, ++, +, +, +: Positive. *: Pink square means sensitive and white square means resistant in the antibiotic susceptibility column

was detected in 186 (92.5%), activity in 106 (52.7%), intestinal metaplasia in 14 (7%), and atrophy in 11 (5.5%) of the samples at different rates, all the samples were negative for dysplasia (Table 3, Fig. 3).

Virulence gene distributions of the *H. pylori* isolates

H. pylori isolates exhibited 8 different genetic profiles (A to H) in terms of the virulence genes. Thus, A, C, B, D, G, H, E and F genetic profiles were detected in 23 (37.7%), 18 (29.5%), 9 (14.8%), 4 (6.6%), 3 (4.9%), 2 (3.3%), 1 (1.6%) and 1 (1.6%) isolate, respectively. The most common virulence gene was *cagA* at the rate of 93.4%. This was followed by the *vacAm2*, *vacAs1*, *vacAs2*, and *vacAm1* with a rate of 83.6%, 62.3%, 26.2% and 23%, respectively. The most common combination among the *vacA* gene alleles was *vacAs1m2* (47.5%), while the lowest was *vacAs2m1* (3.3%). Only one isolate carried all gene alleles tested (genetic profile F) (Table 3, Fig. 3).

No statistically significant relationship could be established between the histopathological examination (inflammation, activity, intestinal metaplasia,

atrophy) and the genetic profiles of the *H. pylori* isolates ($P > 0.05$) (Table 3).

ERIC-PCR findings

A total of 26 different ERIC-PCR profiles (Cluster I (C1-I) to Cluster XXVI (C1-XXVI)) by taking 80% similarity coefficient were obtained. Cluster 26 was the largest cluster containing nine *H. pylori* isolates (Table 4, Fig. 3).

The #179A and #179B isolates obtained from the patient #179 showed different antibiotic susceptibility, virulence gene profiles, and ERIC-PCR profiles. So, they were considered as two different *H. pylori* isolates (Fig. 3).

The #16A and #16B isolates obtained from the patient #16 had different antibiotic susceptibility characteristics and virulence gene profiles, but similar ERIC-PCR profiles. Therefore, they were assumed two different isolates (Fig. 3).

Table 3 The relationship of the current *H. pylori* genotypes with the histopathological analysis findings

Genetic profile	Virulence gene combination	Histopathology											
		Inflammation		Activity		Intestinal metaplasia		Atrophy		Dysplasia		Hp positivity	
		–	+	–	+	–	+	–	+	–	+	–	+
A (n: 23)	<i>cagA</i> +/ <i>vacAm1</i> –/ <i>vacAm2</i> +/ <i>vacAs1</i> +/ <i>vacAs2</i> –	2	19	4	17	18	3	19	2	21	–	6	15
B (n: 9)	<i>cagA</i> +/ <i>vacAm1</i> +/ <i>vacAm2</i> –/ <i>vacAs1</i> +/ <i>vacAs2</i> –	–	9	1	8	9	–	9	–	9	–	2	7
C (n: 18)	<i>cagA</i> +/ <i>vacAm1</i> –/ <i>vacAm2</i> +/ <i>vacAs1</i> –/ <i>vacAs2</i> +	–	17	3	14	17	–	17	–	17	–	5	12
D (n: 4)	<i>cagA</i> –/ <i>vacAm1</i> –/ <i>vacAm2</i> +/ <i>vacAs1</i> –/ <i>vacAs2</i> +	–	4	–	4	4	–	4	–	4	–	1	3
E (n: 1)	<i>cagA</i> +/ <i>vacAm1</i> +/ <i>vacAm2</i> –/ <i>vacAs1</i> –/ <i>vacAs2</i> +	–	1	–	1	1	–	1	–	1	–	–	1
F (n: 1)	<i>cagA</i> +/ <i>vacAm1</i> +/ <i>vacAm2</i> +/ <i>vacAs1</i> +/ <i>vacAs2</i> +	–	1	–	1	1	–	1	–	1	–	1	–
G (n: 3)	<i>cagA</i> +/ <i>vacAm1</i> +/ <i>vacAm2</i> +/ <i>vacAs1</i> +/ <i>vacAs2</i> –	–	3	1	2	2	1	2	1	3	–	1	2
H (n: 2)	<i>cagA</i> +/ <i>vacAm1</i> –/ <i>vacAm2</i> +/ <i>vacAs1</i> +/ <i>vacAs2</i> +	–	2	–	2	2	–	2	–	2	–	–	2
<i>P</i> value		0.8		0.9		0.4		0.4		ND		0.7	
Total*		2	56	9	49	54	4	55	3	58	–	16	42

+Positive, –Negative, Hp: *H. pylori*, *: Three culture-positive samples did not have a histopathological analysis report

Table 4 Distribution of *H. pylori* isolates according to ERIC-PCR motifs and genetic profiles

ERIC-PCR profile	Symbol in dendrogram	Number of the strains	Virulence gene profiles
Cluster 1	CI-I	3	A, B, C
Cluster 2	CI-II	1	A
Cluster 3	CI-III	1	D
Cluster 4	CI-IV	2	C
Cluster 5	CI-V	5	A, B, C
Cluster 6	CI-VI	1	A
Cluster 7	CI-VII	2	A
Cluster 8	CI-VIII	1	B
Cluster 9	CI-IX	1	D
Cluster 10	CI-X	2	A, C
Cluster 11	CI-XI	1	C
Cluster 12	CI-XII	2	A, C
Cluster 13	CI-XIII	3	B, C, H
Cluster 14	CI-XIV	1	I
Cluster 15	CI-XV	1	C
Cluster 16	CI-XVI	5	A, B, C, D
Cluster 17	CI-XVII	1	C
Cluster 18	CI-XVIII	2	A, G
Cluster 19	CI-XIX	2	A, C
Cluster 20	CI-XX	1	A
Cluster 21	CI-XXI	5	A, B, C, G
Cluster 22	CI-XXII	1	A
Cluster 23	CI-XXIII	4	A, C
Cluster 24	CI-XXIV	2	A, F
Cluster 25	CI-XXV	2	A
Cluster 26	CI-XXVI	9	A, B, C, D, E, G, H

According to these findings, the patients #179 and #16 were evaluated as co-infected with different *H. pylori* subtypes (Fig. 3).

Discussion

In the present study, the overall prevalence of *H. pylori* was determined as 63% in adult dyspeptic patients by invasive tests. While this rate is compatible with the previously reported study results both Türkiye and developing countries (40.4 to 86%) (Akar et al. 2021; Dorji et al. 2014; Erkut et al. 2020; Hamrah et al. 2017), it is considerably higher than the rates in developed countries (17 to 34%) (Duggan et al. 2009; Sarnelli et al. 2003). The prevalence of *H. pylori* was determined as 44.6–82.1% by tissue PCR and histopathological examination in the previous

studies in the region where this study was conducted (Adalı et al. 2017; Önder et al. 2016; Tarhane et al. 2019). Unlike the studies mentioned above, cultural method was also performed for isolation of the *H. pylori* in our study. Among these invasive tests, the tissue PCR was found to be highly sensitive (84%) and specific (100%) method which is in line with the literature (Lage et al. 1995; Uyanık et al. 2007).

CH, AC, MZ, TC, LE, and RI are the most commonly used antibiotics in the eradication of *H. pylori* infections worldwide (O'Connor et al. 2019; Sezgin et al. 2019). RI is an important antibiotic used for the treatment of tuberculosis (Grobbelaar et al. 2019). So, RI is not preferred in the treatment of *H. pylori* infection in countries where tuberculosis is prevalent (Akar et al. 2021). In addition, only one study has been reported on the susceptibility of RI to *H. pylori* in Türkiye so far (Akar et al. 2021). Increasing

resistance to the antibiotics used in the treatment of *H. pylori* still poses an important problem. Indeed, worldwide resistance rates to CH, LE, MZ, RI, AC and TC used in the eradication of *H. pylori* vary between 5–92.7%, 8–85.4%, 27.1–65.9%, 0–33%, 0–10% and 0–14%, respectively (Biernat et al. 2014; Khademi and Sahebkar 2020; Kulsunti Wong et al. 2008; Kuo et al. 2017; Kuo et al. 2021; Macías-García et al. 2017; Martinez-Gonzalez et al. 2019; Park et al. 2020; Regnath et al. 2017; Savoldi et al. 2018). In our country, these rates were determined as 8.8–50%, 11.7–62%, 18.2–36%, 0–9.1% and 0–2.9% for CH, MZ, LE, TC and AC, respectively (Caliskan et al. 2015; Cirak et al. 2007; Çağdaş et al. 2012; Erkut et al. 2020; Kocazeybek et al. 2019; Yula et al. 2013). In addition, Akar et al. (2021) recently indicated to a decrease in clarithromycin resistance (19%), an increase in levofloxacin and metronidazole resistance, and reported the first presence of rifampicin resistance (12%) in *H. pylori*. The resistance rates obtained in our study were consistent with the results of the study recently conducted by Akar et al. (2021) in our country.

Ten (16.4%) of the isolates obtained in the current study were found to be susceptible to all antibiotics. While 51 (83.6%) of the *H. pylori* isolates were resistant to at least one antibiotic, the number of MDR isolates was 28 (45.9%). It is seen that the MDR rate obtained in our study is higher than the MDR rate (2.5–26%) reported in the literature (Akar et al. 2021; Biernat et al. 2014; Kulsunti Wong et al. 2008). It is thought that this difference may be related to the number of resistant antibiotics taken into account in MDR description.

Helicobacter pylori plays an important role in the development of chronic gastritis, gastric atrophy, intestinal metaplasia, dysplasia, and gastric cancer (Correa et al. 2012). In our study, the rates of chronic gastritis, intestinal metaplasia, gastric atrophy, and gastric cancer in histopathological examination were 59.6%, 7%, 5.5%, and 4.3%, respectively. These data were consistent with the literature (Akar et al. 2021; Ekesbo et al. 2006) except for no case of dysplasia.

In this study, the most common virulence gene was *cagA* (93.4%) and the least frequently was *vacAm1* (23%). These rates are consistent with the results of the previous studies (Kishk et al. 2021; Parsonnet et al. 1997; Oliveire et al. 2021; Akar et al. 2022). In the current study, the *H. pylori* isolates exhibited 8

different genetic profiles (A–H) according to the *cagA* and *vacA* gene combinations (Table 3, Fig. 3). Simultaneous positivity of *cagA*, *vacAm2* and *vacAs1* genes (genetic profile A) was detected in the present study likewise the other studies (Akar et al. 2022; Soltermann et al. 2007; Falsafi et al. 2015). No significant relationship was found between the *H. pylori* virulence gene combinations (Table 3) and histopathological findings, as in many studies (Kim et al. 2001; Zhang et al. 2001). The major obstacle to establishing a relationship, if any, between the virulence gene combinations and histopathological findings in the current study is that the number of strains included in the virulence gene combinations is not sufficient for statistical analysis. Nevertheless, an association between gastroduodenal diseases and virulence genes on an individual basis has been reported (Akar et al. 2022; Cover et al. 2005; Matsunari et al. 2012; Wattanawongdon et al. 2023). Similar to these reports, *cagA*, *vacAm2* and *vacAs1* genes were detected in either all (metaplasia and atrophy) or most (inflammation and activity) other existing histopathological findings in our study, excluding dysplasia. However, *vacAm1* and *vacAs2* positivity of the present study was generally found to be independent variables from histopathological findings.

ERIC-PCR is an advantageous molecular method used in the genotyping of *H. pylori* isolates which have genetic heterogeneity (Hussain et al. 2004; Finger et al. 2006). Fingerprints generated by ERIC-PCR indicate that ERIC sequences are also present in *H. pylori* strains (Thoreson et al. 2000; Hussain et al. 2004; Finger et al. 2006). In our study, 26 profiles (cluster 1–cluster 26) were obtained as a result of ERIC-PCR analysis of the *H. pylori* isolates, and the isolates showed a high heterogeneity (Table 4, Fig. 3). A similar heterogeneity was also observed in the virulence gene structures and antibiotic resistance profiles of the *H. pylori* isolates. So, there was no relation between the ERIC-PCR motifs and the bacterial characteristics such as the presence of the virulence gene or antibiotic resistance (Fig. 3). The high heterogeneity in ERIC-PCR profiles was due to the fact that the ERIC-PCR primers amplified regions between the 127 bp long repetitive defective palindromes (ERIC sequences) and the positions of these palindromes are variable (Wilson and Sharp 2006). The presence or diversity of inter-palindromes in bacteria cannot be interpreted as the transcription of the

virulence factor or the antimicrobial resistance gene. As a matter of fact, variations were detected in terms of virulence gene distribution and antibiotic resistance even within Cluster 5, 16, 21 and 26 belonging to this study, which contained more strains than other ERIC-PCR profiles. While genetic profiles A and C naturally appear in almost all ERIC-PCR profiles due to their numerical superiority, the main difference between these profiles is the variability in the *s1* and *s2* alleles of the *vacA* gene. Of these, only the presence of *vacAs1* was found to be proportionally higher in histopathological findings and may have clinical significance in terms of peptic ulcer and gastric adenocarcinoma, as reported in other studies (Idowu et al. 2019; Chang et al. 2018). No correlation was found between ERIC-PCR motifs and histopathological findings even endoscopic findings (Fig. 3). The reason for this is the low frequency detection for these variables that will not be sufficient for statistical analysis. In addition, the situation that we could not reconcile between the bacterial characteristics and ERIC motifs is also valid for histopathological findings. Because the course of infection and gross pathology are directly related to the presence of various virulence factors encoded by the *cagA* and *vacA* genes (Gonzalez Vazquez et al. 2016; Kishk et al. 2021), which we could not associate with the ERIC motifs. Indeed, variations in terms of histopathological findings were detected even within Cluster 5, 16, 21 and 26, which contain more strains than other ERIC-PCR profiles (Fig. 3).

A patient can be infected with different *H. pylori* subtypes at the same time. Detection of this mixed infection is very important in terms of antibacterial treatment (Hussain et al. 2004; Finger et al. 2006). In the present study, dual infections with two different *H. pylori* subtypes were detected simultaneously in two the patients (#16 and #179). Therefore, it is essential to evaluate colonies with different morphological characteristics in cultural screening and to perform antibacterial susceptibility tests separately for each *H. pylori* eradication.

Conclusions

In this study, it was observed a high prevalence of *H. pylori* in adult dyspeptic patients. Also, high resistance to the antibiotics frequently used in the

eradication and significant genotypic heterogeneity in the *H. pylori* isolates were detected. It is thought that the detection currently of such markers for each region will play an important role in the eradication of *H. pylori*.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by F.B., E.K., S.T., H.E.O., T.A. and I.B.S. F.B., M.A. and T.K. performed statistical analysis. The first draft of the manuscript was written by F.B., O.C., S.A., S.O., F.A., and all authors commented on previous versions of the manuscript. All authors had full access to the data and participated in the decision to submit for publication. All authors approved the final version of the article, including the authorship list.

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

Declarations

Conflict of interest All other authors declare no conflicts of interest.

References

- Adalı Y, Eroğlu HA, Güvendi GF (2017) Endoscopic gastric biopsy results: Kars Province. *Kafkas J Med Sci* 7:47–52
- Akar M, Aydın F, Kayman T, Abay S, Karakaya E (2021) Detection of *Helicobacter pylori* by invasive tests in adult dyspeptic patients and antibacterial resistance to six antibiotics, including rifampicin in Turkey. Is clarithromycin resistance rate decreasing? *Turk J Med Sci* 51:1445–1464
- Akar M, Kayman T, Abay S, Solakoğlu T, Karakaya E et al (2022) Clinical relevance of virulence genes in *Helicobacter pylori* isolates recovered from adult dyspeptic patients in Turkey. *Indian J Med Microbiol* 40:258–262
- Arsilan N, Yılmaz Ö, Demiray-Gürbüz E (2017) Importance of antimicrobial susceptibility testing for the management of eradication in *Helicobacter pylori* infection. *World J Gastroenterol* 23:2854–2869
- Atherton JC, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ et al (1995) Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types

- with cytotoxin production and peptic ulceration. *J Biol Chem* 270:17771–17777
- Biernat MM, Poniewierka E, Błaszczuk J, Czapla L, Kempniński R et al (2014) Antimicrobial susceptibility of *Helicobacter pylori* isolates from Lower Silesia, Poland. *Arch Med Sci* 10:505–509
- Boyanova L, Yordanov D, Gergova G, Markovska R, Mitov I (2010) Association of *iceA* and *babA* genotypes in *Helicobacter pylori* strains with patient and strain characteristics. *Antonie Van Leeuwenhoek* 98:343–350
- Brennan DE, Dowd C, O'Morain C, McNamara D (2018) Can bacterial virulence factors predict antibiotic resistant *Helicobacter pylori* infection? *World J Gastroenterol* 24:971–981
- Broutet N, Marais A, Lamouliatte H, Mascarel AD, Samoyeau R et al (2001) *CagA* status and eradication treatment outcome of anti-*Helicobacter pylori* triple therapies in patients with nonulcer dyspepsia. *J Clin Microbiol* 39:1319–1322
- Çağdaş U, Otağ F, Tezcan S, Sezgin O, Aslan G et al (2012) Detection of *Helicobacter pylori* and antimicrobial resistance in gastric biopsy specimens. *Mikrobiyol Bul* 46:398–409
- Caliskan R, Tokman HB, Erzin Y, Saribas S, Yuksel P et al (2015) Antimicrobial resistance of *Helicobacter pylori* strains to five antibiotics, including levofloxacin, in Northwestern Turkey. *Rev Soc Bras Med Trop* 48:278–284
- Chang WL, Yeh YC, Sheu BS (2018) The impacts of *H. pylori* virulence factors on the development of gastroduodenal diseases. *J Biomed Sci* 25:68
- Cirak MY, Engin D, Unal S, Karakan T (2007) Monitoring of *Helicobacter pylori* resistance to clarithromycin by real-time PCR assay in gastric biopsy specimens over a 4-year period in Turkey. In: 20th International Workshop on Helicobacter and Related Bacteria in Chronic Digestive Inflammation, İstanbul, Türkiye
- Correa P, Piazzuelo MB (2012) The gastric precancerous cascade. *J Dig Dis* 13:2–9
- Cover TL, Blanke SR (2005) *Helicobacter pylori vacA*, a paradigm for toxin multifunctionality. *Nat Rev Microbiol* 3:320–332
- Dorji D, Dendup T, Malaty HM, Wangchuk K, Yangzom, et al (2014) Epidemiology of *Helicobacter pylori* in Bhutan: the role of environment and Geographic location. *Helicobacter* 19:69–73
- Duggan AE, Elliott CA, Miller P, Hawkey CJ, Logan RFA (2009) Clinical trial: a randomized trial of early endoscopy, *Helicobacter pylori* testing and empirical therapy for the management of dyspepsia in primary care. *Aliment Pharmacol Ther* 29:55–68
- Ekesbo R, Toth E, Fork FT, Held M, Nilsson I et al (2006) Chronic *Helicobacter pylori* infection in a population in southern Sweden analysed by histopathology, immunoblot and ELISA serology. *Eur J Gastroenterol Hepatol* 18:589–593
- Elviss NC, Owen RJ, Xerry J, Walker AM, Davies K (2004) *Helicobacter pylori* antibiotic resistance patterns and genotypes in adult dyspeptic patients from a regional population in North Wales. *J Antimicrob Chemother* 54:435–440
- Erkut M, Uzun DY, Kakhkaya N, Fidan S, Yoğun Y et al (2020) Sociodemographic characteristics and clinical risk factors of *Helicobacter pylori* infection and antibiotic resistance in the Eastern Black Sea region of Turkey. *Turk J Gastroenterol* 31:221–233
- EUCAST (2019) MIC distributions and the setting of epidemiological cutoff (ECOFF) values. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_SOPs/EUCAST_SOP_10.1_MIC_distributions_and_epidemiological_cut-off_value__ECOFF__setting_20191130.pdf. Accessed 1 Aug 2022
- Fallone CA, Loo VG, Lough J, Barkun AN (1997) Hematoxylin and eosin staining of gastric tissue for the detection of *Helicobacter pylori*. *Helicobacter* 2:32–35
- Falsafi T, Khani A, Mahjoub F, Asgarani E, Sotoudeh N (2015) Analysis of *vacA/cagA* genotypes/status in *Helicobacter pylori* isolates from Iranian children and their association with clinical outcome. *Turk J Med Sci* 45:170–177
- Fendri I, Ben Hassena A, Grosset N, Barkallah M, Khannous L et al (2013) Genetic diversity of food-isolated *Salmonella* strains through Pulsed Field Gel Electrophoresis (PFGE) and Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR). *PLoS ONE* 8:e81315
- Finger SA, Velapattino B, Kosek M, Santivañez L, Dailidene D et al (2006) Effectiveness of enterobacterial repetitive intergenic consensus PCR and random amplified polymorphic DNA fingerprinting for *Helicobacter pylori* strain differentiation. *Appl Environ Microbiol* 72:4713–4716
- Gonzalez Vazquez R, Córdova Espinoza MG, Escamilla Gutiérrez A, Morales-Méndez I, Ochoa-Pérez SA et al (2016) Frequency of virulence genes in mixed infections with *Helicobacter pylori* strains from a Mexican population. *Rev Gastroenterol Mex* 81:11–20
- Grobbelaar M, Louw GE, Sampson SL, van Helden PD, Donald PR, Warren RM (2019) Evolution of rifampicin treatment for tuberculosis. *Infect Genet Evol* 74:103937
- Hamrah MH, Hamrah MS, Hassan Hamrah M, Kanda M, Hamrah AE et al (2017) Prevalence of *Helicobacter pylori* infection in dyspeptic patients in Andkhoy Afghanistan. *Asian Pac J Cancer Prev* 18:3123–3127
- Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M et al (2002) Biological activity of the *Helicobacter pylori* virulence factor *cagA* is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci USA* 99:14428–14433
- Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE et al (2017) Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. *Gastroenterol* 153:420–429
- Houf K, De Zutter L, Van Hoof J, Vandamme P (2002) Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. *Appl Environ Microb* 68:2172–2178
- Hussain MA, Kauser F, Khan AA, Tiwari S, Habibullah CM et al (2004) Implications of molecular genotyping of *Helicobacter pylori* isolates from different human populations by genomic fingerprinting of enterobacterial repetitive intergenic consensus regions for strain identification and geographic evolution. *J Clin Microbiol* 42:2372–2378

- Idowu A, Mzukwa A, Harrison U, Palamides P, Haas R et al (2019) Detection of *Helicobacter pylori* and its virulence genes (*cagA*, *dupA*, and *vacA*) among patients with gastro-duodenal diseases in Chris Hani Baragwanath Academic Hospital, South Africa. *BMC Gastroenterol* 19:73
- İrvem A, Kolaylı F, Hülal S (2013) Comparison of *Helicobacter pylori* virulence genes and endoscopic findings in patients with dyspeptic complaints. *Türk Mikrobiyol Cem Derg* 43:104–111
- Khademi F, Sahebkar A (2020) An updated systematic review and meta-analysis on the *Helicobacter pylori* antibiotic resistance in Iran. *Microb Drug Resist* 26:1186–1194
- Kim SY, Woo CW, Lee YM, Son BR, Kim JW et al (2001) Genotyping *cagA*, *vacA* subtype, *iceA1*, and *babA* of *Helicobacter pylori* isolates from Korean patients, and their association with gastro-duodenal diseases. *J Korean Med Sci* 16:579–584
- Kishk RM, Soliman NM, Anani MM, Nemr N, Salem A et al (2021) Genotyping of *Helicobacter pylori* virulence genes *cagA* and *vacA*: regional and national study. *Int J Microbiol* 5540560:1–7
- Kocazeybek B, Sakli MK, Yuksel P, Demirci M, Caliskan R et al (2019) Comparison of new and classical point mutations associated with clarithromycin resistance in *Helicobacter pylori* strains isolated from dyspeptic patients and their effects on phenotypic clarithromycin resistance. *J Med Microbiol* 68:566–573
- Kulsunti Wong P, Chomvarin C, Chaicumpar K, Namwat W (2008) Antimicrobial susceptibility of *Helicobacter pylori* isolated from gastric biopsies in dyspeptic patients. *Southeast Asian J Trop Med Public Health* 39:1102–1109
- Kuo CJ, Lee CH, Chang ML, Lin CY, Lin WR et al (2021) Multidrug resistance: The clinical dilemma of refractory *Helicobacter pylori* infection. *J Microbiol Immunol Infect* 54:1184–1187
- Kuo YT, El-Omar EM, Wu JY, Leow AHR, Goh KL et al (2017) Primary antibiotic resistance in *Helicobacter pylori* in the Asia-Pacific region: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2:707–715
- Lage AP, Godfroid E, Fauconnier A, Burette A, Butzler JP et al (1995) Diagnosis of *Helicobacter pylori* infection by PCR: comparison with other invasive techniques and detection of *cagA* gene in gastric biopsy specimens. *J Clin Microbiol* 33:2752–2756
- Lu JJ, Perng CL, Shyu RY, Chen CH, Lou Q et al (1999) Comparison for five PCR methods for detection of *Helicobacter pylori* in gastric tissues. *J Clin Microbiol* 37:772–774
- Macías-García F, Llovo-Taboada J, Díaz-López M, Bastón-Rey I, Domínguez-Muñoz JE (2017) High primary antibiotic resistance of *Helicobacter pylori* strains isolated from dyspeptic patients: a prevalence cross-sectional study in Spain. *Helicobacter* 22:6
- Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ et al (2017) European Helicobacter and Microbiota Study Group and Consensus Panel. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 66:6–30
- Manxhuka-Kerliu S, Telaku S, Devolli-Disha E, Ahmetaj H, Sahatciu-Meka V et al (2009) *Helicobacter pylori* gastritis updated Sydney classification applied in our material. *Prilozi* 30:45–60
- Martinez-Gonzalez B, Georgopoulos S, Michopoulos S, Karayiannis Y, Liatsos C et al (2019) Multicenter survey of antimicrobial resistance in *Helicobacter pylori* isolates in Greece-Trends of resistance 1998–2018. *Cancer* 24:e12647–e12647
- Matsunari O, Shiota S, Suzuki R, Watada M, Kinjo N, Murakami K, Fujioka T, Kinjo F, Yamaoka Y (2012) Association between *Helicobacter pylori* virulence factors and gastro-duodenal diseases in Okinawa, Japan. *J Clin Microbiol* 50:2542
- Miernyk KM, Bruden D, Rudolph KM, Hurlburt DA, Sacco F et al (2020) Presence of *cagPAI* genes and characterization of *vacA* s, i and m regions in *Helicobacter pylori* isolated from Alaskans and their association with clinical pathologies. *J Med Microbiol* 69(2):218–227
- O'Connor A, Liou JM, Gisbert JP, O'Morain C (2019) Review: treatment of *Helicobacter pylori* infection 2019. *Helicobacter* 24(Suppl 1):e12640
- Oliveira AKS, Silva LLL, Miguel MP, Blanco AJV, Carneiro LC et al (2021) *Helicobacter pylori* *cagA* virulence gene and severe esogastro-duodenal diseases: Is there an association? *Arq Gastroenterol* 58:468–475
- Önder T, Hasbal NB, Kahraman Ş, Anuk T (2016) The relationship between *Helicobacter pylori* infection and sex, blood groups and rhesus factor at the patients with chronic gastritis. *İKSST Derg* 8:93–96
- Oth L, Wilson M, Fernández H, Oth C, Toledo C et al (2011) Isolation of *Helicobacter pylori* in gastric mucosa and susceptibility to five antimicrobial drugs in Southern Chile. *Braz J Microbiol* 42:442–447
- Ozaydin N, Turkyilmaz SA, Cali S (2013) Prevalence and risk factors of *Helicobacter pylori* in Turkey: a nationally-representative, cross-sectional, screening with the ¹³C-Urea breath test. *BMC Public Health* 13:1215
- Ozbey G, Aygun C (2012) Prevalence of genotypes in *Helicobacter pylori* isolates from patients in Eastern Turkey and the association of these genotypes with clinical outcome. *Braz J Microbiol* 43:1332–1339
- Park JY, Shin TS, Kim JH, Yoon HJ, Kim BJ et al (2020) The prevalence of multidrug resistance of *Helicobacter pylori* and its impact on eradication in Korea from 2017 to 2019: a single-center study. *Antibiotics (basel)* 9:646
- Parsonnet J, Friedman GD, Orentreich N, Vogelstein H (1997) Risk for gastric cancer in people with *cagA* positive or *cagA* negative *Helicobacter pylori* infection. *Gut* 40:297–301
- Regnath T, Raecke O, Enninger A, Ignatius R (2017) Increasing metronidazole and rifampicin resistance of *Helicobacter pylori* isolates obtained from children and adolescents between 2002 and 2015 in southwest Germany. *Helicobacter* 22:1
- Roesler BM, Rabelo Gonçalves EMA, Zeitune JMR (2014) Virulence factors of *Helicobacter pylori*: a review. *Clin Med Insights Gastroenterol* 7:9–17
- Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF (2000) Histological identification of *Helicobacter pylori*: comparison of staining methods. *J Clin Pathol* 53:756–759
- Sarnelli G, Cuomo R, Janssens J, Tack J (2003) Symptom patterns and pathophysiological mechanisms in dyspeptic

- patients with and without *Helicobacter pylori*. Dig Dis Sci 48:2229–2236
- Saticioglu IB, Duman M, Wiklund T, Altun S (2018) Serological and genetic characterization of *Flavobacterium psychrophilum* isolated from farmed salmonids in Turkey. J Fish Dis 41:1899–1908
- Savoldi A, Carrara E, Graham DY (2018) Prevalence of antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis in World Health Organization regions. Gastroenterol 155:1372–1382
- Sezgin O, Aydın MK, Özdemir AA, Kanık AE (2019) Standard triple therapy in *Helicobacter pylori* eradication in Turkey: systematic evaluation and meta-analysis of 10-year studies. Turk J Gastroenterol 30:420–435
- Soltermann A, Koetzer S, Eigenmann F, Komminoth P (2007) Correlation of *Helicobacter pylori* virulence genotypes *vacA* and *cagA* with histological parameters of gastritis and patient's age. Mod Pathol 20:878–883
- Sugimoto M, Yamaoka Y (2009) Virulence factor genotypes of *Helicobacter pylori* affect cure rates of eradication therapy. Arch Immunol Ther Exp (warsz) 57:45–56
- Tarhane S, Anuk T, Gülmez Sağlam A, Özcan HE et al (2019) *Helicobacter pylori* positivity and risk analysis in patients with abdominal pain complaints. Mikrobiyol Bul 53:262–273
- Thoreson AC, Hosseini N, Svennerholm AM, Bölin I (2000) Different *Helicobacter pylori* strains colonize the antral and duodenal mucosa of duodenal ulcer patients. Helicobacter 5:69–78
- Tummuru MK, Cover TL, Blaser MJ (1993) Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect Immun 61:1799–1809
- Uyanık MH, Aktaş O, Özbek A, Yılmaz Ö, Ayyıldız A (2007) Identification of *Helicobacter pylori*: comparison of methods. İnfeksiyon Derg 21:123–128
- Versalovic J, Koeuth T, Lupskil JR (1991) Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 19:6823–6831
- Wattanawongdon W, Simawaranon Bartpho T, Tongtawee T (2023) Relationship between *Helicobacter pylori* virulence genes and gastroduodenal disease. J Int Med Res 51:3000605231161465
- Wijetunge DS, Dunn P, Wallner-Pendleton E, Lintner V, Lu H et al (2012) Fingerprinting of poultry isolates of *Enterococcus cecorum* using three molecular typing methods. J Vet Diagn 24:1166–1171
- Wilson LA, Sharp PM (2006) Enterobacterial repetitive intergenic consensus (ERIC) sequences in *Escherichia coli*: evolution and implications for ERIC-PCR. Mol Biol Evol 23:1156–1168
- Yula E, Nagiyev T, Kaya OA, Inci M, Celik MM et al (2013) Detection of primary clarithromycin resistance of *Helicobacter pylori* and association between *cagA* (+) status and clinical outcome. Folia Microbiol (praha) 58:141–146
- Zhang Y, Liu H, Zhou K (2001) Lack of correlation of *vacA* genotype, *cagA* gene of *Helicobacter pylori* and their expression products with various gastroduodenal diseases. Chin Med J (engl) 114:703–706

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