GMJ 2024;35:351-356

DOI: http://dx.doi.org/10.12996/gmj.2024.3624

Pathogenic and Genetic Characteristics of *Helicobacter Pylori*, and its Relationship with Drug-Resistance

Helicobacter pylori'nin Patojenik ve Genetik Özellikleri ve İlaç Direnciyle İlişkisi

Nilgün Uyduran Ünal[1], Adnan Barutçu[1], Toğrul Nagiyev[2], Mehmet Ağın[3], Tülay Kandemir[2], Oğuz Üsküdar[4], Figen Doran[5], Fatih Köksal[2], Gökhan Tümgör[3]

[1]Department of Pediatrics, Çukurova University Faculty of Medicine, Adana, Türkiye
[2]Department of Medical Microbiology, Çukurova University Faculty of Medicine, Adana, Türkiye
[3]Department of Pediatric Gastroenterology, Çukurova University Faculty of Medicine, Adana, Türkiye
[4]Department of Gastroenterology, Çukurova University Faculty of Medicine, Adana, Türkiye
[5]Department of Pathology, Çukurova University Faculty of Medicine, Adana, Türkiye

ABSTRACT

Objective: This study aimed to determine the epidemiological characteristics of *Helicobacter pylori (H. pylori)* infection at the genotype level in patients presenting with dyspeptic complaints, and to show the distribution of virulence factors, importance of intrafamilial transmission, as well as the distribution of resistance to macrolide and quinolone antibiotics.

Methods: The study comprised 110 patients with dyspeptic complaints who were admitted to our hospital between January 13, 2015, and December 31, 2016. Through histopathology, culture, and glmM-polymerase chain reaction (PCR) techniques, we detected *H. pylori*. The *vacA*, *cagA*, and *cagE* genes were determined in patients with positive PCR results were positive.

Results: *H. pylori* strains and clinical results were not found to be significantly correlated in the study. Both genetic variants *A2142G* and *A2143G* were discovered to be present in the individuals. Eight patients had clarithromycin resistance (34.7%). All patients with a positive A2142G mutation and 55% of patients with a positive A2143G mutation were found to have clarithromycin resistance. Levofloxacin resistance was present in only one (4.3%) patient who could produce *H. pylori* in culture.

Conclusion: Approximately 1/3 of the children with dyspeptic complaints were positive for *H. pylori* infection. The most common genotype was observed to be vacAs2. Even individuals with at least one of the genetic mutations A2142G and A2143G have the potential for antibiotic resistance. High resistance was found against clarithromycin in the standard triple therapy regimen used in children for treating *H. pylori* infection.

Keywords: *Helicobacter pylori*, children, virulence, antibiotic resistance, endoscopy, genetic

ÖZ

Amaç: Çalışma, dispeptik şikayetleri olan çocuk hastalarda *Helicobacter pylori (H. pylori)* enfeksiyonlarının sıklığını belirlemeyi ve aile içi geçişin önemini, virülans faktörlerinin dağılımını, ayrıca kinolon ve makrolid grubu antibiyotiklere karşı direncin epidemiyolojik özelliklerini genotip düzeyinde belirleyerek ortaya koymayı amaçlamaktadır.

Yöntemler: Çalışmaya 13 Ocak 2015-1 Aralık 2016 tarihleri arasında hastanemize dispeptik şikayetlerle başvuran 110 hasta dahil edildi. *H. pylori*; histopatolojik, kültür ve glmM-polimeraz zincir reaksiyonu (PZR) yöntemleri ile araştırıldı. PZR'si pozitif saptanan hastalarda *vacA, cagA* ve *cagE* genlerinin tespiti yapıldı.

Bulgular: Çalışmada *H. pylori* suşları ile klinik bulgular arasında anlamlı bir ilişki saptanmamıştır. Etkilenen hastalarda hem A2142G hem de A2143G'nin genetik mutasyonları pozitif bulundu. Sekiz hastada (%34,7) klaritromisin direnci saptandı. A2142G mutasyonu pozitif saptanan hastaların tümünde, A2143G mutasyonu pozitif saptananların %55'inde klaritromisin direnci gözlendi. Kültürde *H. pylori* üretebilen hastaların sadece birinde (%4,3) levofloksasin direnci gözlendi.

Sonuç: Dispeptik şikayetleri olan çocukların yaklaşık 1/3'ünde *H. pylori* enfeksiyonu pozitif saptanmıştır. En çok görülen genotip vacAs2 olduğu gözlenmiştir. A2142G ve A2143G genetik mutasyonlarından en az birine sahip olmak bile antibiyotik direnci potansiyeli taşımaktadır. *H. pylori* enfeksiyonu tedavisinde çocuklarda kullanılan standart üçlü tedavi rejiminde bulunan klaritromisine karşı yüksek direnç saptanmıştır.

Anahtar Sözcükler: *Helicobacter pylori*, çocuklar, virülans, antibiyotik direnci, endoskopi, genetik

Address for Correspondence/Yazışma Adresi: Adnan Barutçu, MD, Department of Pediatrics, Çukurova University Faculty of Medicine, Adana, Türkiye
E-mail / E-posta: adnan_barutcu@hotmail.com
ORCID ID: orcid.org/0000-0001-8930-1122

Received/Geliş Tarihi: 20.07.2022
Accepted/Kabul Tarihi: 10.06.2024

INTRODUCTION

Helicobacter pylori (H. pylori) is the most important cause of gastroduodenal pathology, ranging from non-ulcer dyspepsia to gastric ulcer and mucosa-associated lymphoid tissue lymphoma, to atrophic gastritis to lower-gastrointestinal system (GIS) carcinomas (1-4). Epidemiological studies have suggested that *H. pylori* is usually transmitted from the family in childhood; more than one strain initiated the infection in the first transmission, but one of the strains adapted to the gastric mucosa in the subsequent period (3).

Although many factors belonging to bacteria and the host have been suggested in the possible relationship between gastroduodenal pathology and *H. pylori*, it can be said most crucial evidence in this context is the clinical cure after *H. pylori* eradication therapy. The frequent occurrence of reactivations due to resistance to various antibiotic regimens applied in recent years in bacteria can be considered the second vital data that strengthens the relationship. Developing more rational treatments will only be possible by identifying colonized strains and knowing the movement of resistance in society by identifying resistant strains.

This study aimed to determine the epidemiological characteristics of *H. pylori* infection at the genotype level in childhood gastroduodenal pathologies. In addition, this study aimed to evaluate the initial period of the *H. pylori*-human relationship, the characteristics of the colonized strain(s), the distribution of virulence factors, the importance of intra-familial transmission, and the primary/secondary resistance distribution to macrolide and quinolone group antibiotics.

MATERIALS AND METHODS

A total of 110 cases admitted with dyspeptic complaints between 13 February 2015 and 1 December 2016 were included in the study. The criteria for inclusion in the study; cases between the ages of 3 and 18 years were identified as cases in which upper GIS endoscopy was planned due to dyspepsia and patients who had not previously received eradication treatment for *H. pylori*. Patients with upper gastrointestinal tract bleeding, patients with a history of gastroduodenal surgery, patients who did not agree to participate in the study, patients with a history of antibiotic and/or proton pump inhibitor use up to 1 month before the study, and patients with chronic diseases were excluded from the study.

After upper GIS endoscopy were performed and the findings were recorded, three biopsy samples were collected from the antrum region of the patients included in the study. The first sample was used for pathological examination, the second was used for *H. pylori* isolation in culture medium and for determining antibiotic susceptibility, and the third was used for genotypic examination. The E-test was performed to assess antibiotic susceptibility to *H. pylori* strains isolated in culture, and the minimum inhibitory concentration (MIC) values were determined. In our study, clarithromycin and levofloxacin E-test strips (Biotests, Ankara, Türkiye, and Liofilche, Italy) were used. The MICs were accepted as determined by the European Committee on Antimicrobial Susceptibility Testing in 2016 (5). For genotypic examinations, DNA extraction was performed from gastric biopsy samples, and these extracts were first examined by polymerase chain reaction (PCR) with the help of specific primers to determine the *glmM* gene sequence. The samples with positive *glmM* gene were accepted as *H. pylori*-positive, and their DNA extracts were evaluated by PCR for the detection of vacA, cagA, and cagE, PCR-RFLP for the detection of clarithromycin resistance, and DNA Sequence analysis for the detection of levofloxacin resistance. Additionally, to detect familial genetic and pathogenic inheritance, upper GIS endoscopy was performed by an adult gastroenterologist in 7 parents of 6 *H. pylori*-positive cases.

Ethics Committee approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Çukurova University Faculty of Medicine (approval number: 37, date: 05.12.2014). The relatives of all the children included in the study were interviewed. After providing information about the interventional procedures to be applied to patients, the purpose of the research, and where to use the data, verbal and written consent was obtained.

Statistical Analysis

Statistical evaluations were performed using the SPSS 21.0 package program. Descriptive data are presented as percentage distributions. The chi-square test was used to compare the gender distribution, endoscopic and genotypic findings. Fischer's exact test was used to compare categorical variables. The statistical significance level was established as 0.05 in all tests.

RESULTS

The mean age of the cases was 12.2±4.39 years (3-18 years), as 38 were male (34.5%) and 72 were female (65.5%). *H. pylori* was found to be positive in 30 cases (27.3%) by PCR method. 18.8% of *H. pylori*-positive patients were between 3-6 years old, 33.3% were between 7-12 years old, and 25.9% were between 13-18 years old. No statistically significant difference was found between the *H. pylori*-positive and *H. pylori*-negative groups in terms of gender and age (p=0.235, p=0.519, respectively).

Of 30 PCR-positive patients, 29 (96.6%) were found to be vacA positive, and vacAs1+cagA+cagE association was found in 16.6% (Table 1).

Among the virulence factors, cagA was found to be positive in 50% of vacAs1-positive patients and 29.4% of vacAs2-positive patients. When the association between vacA subtypes and cagA was examined, no statistically significant difference was found (p=0.390).

Table 1. Distribution of virulence genes in 30 patients with *H. pylori* detected by PCR method

***Virulence* genes**	**n=30 (%)**
vacA	29 (96.6)
vacAs1	12 (40)
vacAs2	17 (56.6)
cagA	11 (36.7)
cagE	7 (23.3)
vacAs1 + cagA	1 (3.3)
vacAs1 + cagE	2 (6.6)
vacAs1 + cagA + cagE	5 (16.6)
vacAs2 + cagA	5 (16.6)

PCR: Polymerase chain reaction

It was found that 58.3% of the vacAs1-positive patients were cagE-positive. No association was observed between vacAs2 and cagE in any of the patients. When the distribution of virulence genes in *H. pylori* PCR-positive cases was examined, no statistically significant relationship was found (p>0.05).

It was observed that vacAs2, one of the less virulent vacA alleles, was found in all cases with normal histopathology, cagA was positive in 66.6% of these cases, and vacAs1 and cagE were not found in these cases. In patients with active chronic gastritis, the *vacAs1*, *vacAs2*, *cagA*, and *cagE* virulence genes were found to be 44.4%, 51.8%, 33.3%, and 25.9%, respectively.

Antibiogram was performed in 23 (20.9%) cases with positive *H. pylori* culture. Clarithromycin resistance was found in 8 (34.7%) patients, and no significant difference was found between genders in terms of clarithromycin resistance (p=0.342). Of clarithromycin-resistant cases, vacAs1 was found to be positive in 50%, vacAs2 in 36.4%, cagA in 40%, and cagE in 66.7%. However, no statistically significant intergroup difference was observed regarding the relationship between virulence genes and clarithromycin resistance (Table 2). Levofloxacin resistance was observed in only one case (4.3%), and vacAs1 was positive; vacAs2, cagA, and cagE were negative in this case.

When the *A2142G* gene mutation distribution was examined according to the age groups of the patients, it was found that there were no genetic mutations between the ages of 3-6 and the presence of genetic mutations at a rate of 16.7% between the ages of 7-12 and 20% between the ages of 13-18. When the *A2143G* gene mutation distribution was examined by age groups, it was observed with a rate of 33.3% between ages 3-6, 25% between ages 7-12, and 53.3% between ages 13-18. Per age group, no statistically significant difference was found in terms of *A2142G* and *A2143G* gene mutation positivity (p=0.698, p=0.318, respectively).

It was found that all patients with the positive A2142G mutation were clarithromycin-resistant, and clarithromycin resistance was found to be significantly higher than those who were negative (p=0.023). No significant difference was found in clarithromycin resistance between the A2143G mutation-positive patients than the negative ones (p=0.054) (Table 3). Both *A2142G* and *A2143G* genetic mutations were found to be positive in a single patient with levofloxacin resistance.

In our study, endoscopy was performed in 7 parents of 6 PCR-positive patients with clinical symptoms, and different *H. pylori* strains were found in the children and parents (Table 4).

Table 2. The relationship of *H. pylori* virulence genes with clarithromycin resistance

***Virulence* genes**	**Clarithromycin-susceptible, n (%)**	**Clarithromycin-resistant, n (%)**	**p**
vacA (-)	0 (0)	1 (100)	
vacAs1 (+)	3 (50)	3 (50)	0.446
vacAs2 (+)	7 (63.6)	4 (36.4)	
cagA (-)	7 (53.8)	6 (46.2)	1.000*
cagA (+)	3 (60)	2 (40)	
cagE (-)	9 (60)	6 (40)	0.559*
cagE (+)	1 (33.3)	2 (66.7)	

*Fisher exact test.

Table 3. Distribution of *A2142G* and *A2143G* genetic mutations in clarithromycin-susceptible and clarithromycin-resistant patients

Genetics mutations	**Clarithromycin-susceptible, n (%)**	**Clarithromycin-resistant, n (%)**	**p**
A2142G negative	15 (83.3)	3 (16.7)	0.023*
A2142G positive	0 (0)	5 (100)	
A2143G negative	10 (83.3)	2 (16.7)	0.054*
A2143G positive	5 (45)	6 (55)	

*Fisher exact test

DISCUSSION

H. pylori is one of the most common pathogens in humans (6). In addition to virulence factors such as urease activity and motility, immunogenic cross-reactivity in gastric cells plays an essential role in the pathogenesis of *H. pylori* (7). In addition, it is thought that some of the *H. pylori* virulence genes increase the risk of developing gastric lesions, and the pathogenic effect of bacteria virulence factors is affected by many factors belonging to the environment and host (8).

In our country, the number of studies evaluating *H. pylori* virulence genes in children is extremely low. Looking at *vacA* isolates from virulence genes, in 2013, Ozbey et al. (9) reported that 91.8% of vacAs1 isolates in *H. pylori*-positive children, and in 2014, Karabiber et al. (10) reported that 81.6% of them were vacAs1 subtype, and

Table 4. Comparison of PCR-positive patients and their parents' virulence genes and genetic mutations

Patients and parents	**vacAs1**	**vacAs2**	**cagA**	**cagE**	**A2142G**	**A2143G**
Patient A	+	-	-	+	-	-
Mother of patient A	+	-	+	+	-	-
Father of patient A	+	-	+	+	-	-
Patient B	-	+	+	-	-	-
Mother of patient B	-	+	-	-	-	-
Patient C	+	-	-	-	-	+
Father of patient C	+	-	+	-	-	-
Patient D	-	+	+	-	-	-
Mother of patient D	-	+	-	-	-	-
Patient E	+	-	-	-	-	+
Mother of patient E	-	+	-	-	-	+
Patient F	+	-	-	-	-	-
Mother of patient F	-	+	-	-	-	-

PCR: Polymerase chain reaction.

the most common vacA subtype was vacAs1a/m2, at a rate of 32.7%. vacAs1/m1 was the most common allele in 85% of symptomatic children in Venezuela and vacAs1 in 82.5% of asymptomatic children in Brazil (11,12). In a study conducted by Erdoğdu et al. (13) in 120 children, it was found that 70.1% of *H. pylori* isolates had vacAs1a, 2.8% had vacAs1b, and 27.1% had the vacAs2 subtype, and reported that vacA genotype was not associated with endoscopic findings. While the most common genotype in Iranian children is vacAs1/m2; it was found that nodular gastritis is common in endoscopic findings, and this is significantly associated with the presence of vacAm1 (14). In our study, the vacAs1 allele was found to be positive in 40% of *H. pylori*-positive cases, and the vacAs2 allele, which is known to be less virulent, in 56.6%. Compared with these two studies, it was observed that the vacAs2 allele was higher in our study. In our research, no statistically significant difference was found in the evaluation of the vacAs1 and vacAs2 genotypes according to endoscopic findings and histopathological data.

In our study, cagA was found with a rate of 36.7%. In symptomatic children, *H. pylori* cagA strains and serum cagA antibodies are found at the rate of 33% -80% (15-17). In a study by Kato et al. (18) conducted on 25 children with the ulcer, it was reported that cagA was detected in 81.8% of asymptomatic children, 93.3% of patients with gastritis, and 80% of patients with gastric ulcers. In a study conducted by Saltik et al. (19), 45 children were evaluated for abdominal pain and tested only for cagA, which is one of the virulence factors. They found cagA positivity at a rate of 55.6%, and they reported that there was no relationship between the severity of gastrointestinal findings.

When *cagE*, a virulence gene, was examined, 23.3% of the 30 PCR-positive patients in our study were found to be cagE positive. A vacAs1+cagE association was found in 6.6% of these, and a vacAs1+cagA+cagE association was found in 16.6%. Because there were not enough studies on this subject in children, cagE positivity was found at a low rate in our study compared to adult studies conducted in our country, whereas a similar rate of cagE positivity was found when compared to the study conducted by Ozbey et al. (9).

In our study, active chronic gastritis was detected in 90% of *H. pylori* PCR-positive 30 patients, and histopathological evaluation of 14.3% of these patients was found to be normal. Among these *H. pylori* PCR-positive cases, it was observed that all of the vacA alleles with normal histopathology were vacAs2, one of the less virulent vacA alleles, and 66.6% of these patients with normal histopathology had cagA and no vacAs1 and cagE, which play an important role in virulence. In patients with active chronic gastritis, the *vacAs1*, *vacAs2*, *cagA*, and *cagE* virulence genes were found to be 44.4%, 51.8%, 33.3%, and 25.9%, respectively. While less virulent genes were detected in cases with normal histopathological evaluation, the positivity of genes known to be more virulent in active chronic gastritis cases was remarkable.

In countries where clarithromycin, metronidazole, and amoxicillin, which are the first-choice antibiotics, are used extensively in eradication treatment, the development of increasing resistance against these drugs causes treatment failure. Therefore, resistance determination has gained importance in the establishment of treatment protocols (20-22). Although many adult studies aimed at determining antibiotic resistance, there are not enough studies on children. Recent data on clarithromycin resistance in our country are generally obtained from studies conducted in adults, and resistance rates vary between 40.2% and 48% (23-25). The use of macrolide group antibiotics, especially in respiratory tract infections, is one of the most important reasons for the development of clarithromycin resistance. It is thought that clarithromycin resistance is a much more critical problem in developed countries, such as our country, where antibiotic use rates are high, or especially in developed countries where macrolide group antibiotics are prescribed for the treatment of respiratory tract infections (26-28).

In the study of Ozçay et al. (29), which was performed on 102 children diagnosed with *H. pylori* infection by urea breath test, serology, or culture, the rate of clarithromycin resistance was found as 18.1%. Karabiber et al. (10) reported that 51 (52%) of 98 PCR-positive children with *H. pylori* gastritis were culture-positive, and clarithromycin, metronidazole, and amoxicillin resistance were 23.5%, 11.7%, and 3.9%, respectively. In the same study, no relationship was found between the presence of cagA, and clarithromycin and metronidazole resistance. Clarithromycin resistance was found at a rate of 83.3% in cagE-positive patients, while this rate decreased to 16.7% in cagE-negative patients (10). In a study conducted on 118 patients, 52.7% of whom were children, in Spain in 2008, it was reported that the clarithromycin resistance measured by the E-test method was 35.6% (30). It was found that metronidazole and clarithromycin resistance tended to increase among children infected with *H. pylori* in China at rates of 49.2% and 34.9%, respectively (31). In Japan, resistance rates to metronidazole and clarithromycin were found to be 43.3% and 21.9%, respectively (32). Levofloxacin, which is effective against *H. pylori in vitro*, has more limited effects in vivo. Wong et al. (33) reported 18%, and Perna et al. (34) reported 30.3% levofloxacin-resistance. In another study, resistance to fluoroquinolone group drugs, which are not commonly used in children, was shown as 30.8% at the age of 0-6, and it was thought that resistance to these drugs was probably acquired from parents (35). The clarithromycin resistance observed in our study is compatible with studies in the literature. However, more meaningful results can be obtained by increasing the number of patients. If *H. pylori* cannot be destroyed sufficiently due to clarithromycin resistance, which is thought to be caused by unnecessary antibiotic use, triple and sequential treatments containing the fluoroquinolone group instead of the macrolide group are promising for older children. The rapid development of resistance to fluoroquinolone group antibiotics is worrying in this respect (35-38).

H. pylori is a microorganism with a high degree of genetic variability. While some studies reported a relationship between virulence genes and antibiotic resistance, some studies reported no such relationship (10,39,40). In our study, no relationship was found between vacA subtypes, cagA and cagE, and antibiotic resistance.

In the study conducted by Caliskan et al. (41) isolate a total of 98 *H. pylori* strains, 36 (36.7%) patients had clarithromycin resistance, 34 (35.5%) had metronidazole resistance, 29 (29.5%) had levofloxacin resistance, and multiple antibiotic resistance was detected in 19.3% of the patients. In the same study, the A2143G and A2144G genetic mutations were detected in 100% of clarithromycin-resistant strains. In our study, clarithromycin resistance was detected in 100% of the five patients who were found to be positive for the A2142G mutation

and underwent antibiogram, and in 6 (55%) of the patients who were A2143G positive. Both mutations were found in male patients with levofloxacin resistance. Even at least one of these genetic mutations has the potential to become resistant to antibiotics.

A comparison of the *vacAs1*, *vacAs2*, *cagA*, and *cagE* virulence genes and *A2142G* and *A2143G* genetic mutations were made PCR-positive six patients with and seven parents of these patients. It has been shown that the *H. pylori* strains of the patient and their parents are different, and in 4 of the six patients we compared, the *H. pylori* strain with the same vacA allele as their parents was detected, whereas bacteria with different cagE and *cagA* genes were detected in these patients. It is known that the *cagA* and *cagE* genes located on the Cag Pathogenicity Island (cagPAI) of *H. pylori* may be acquired later or that existing genes may be lost in the future. While evaluating the differences in *H. pylori* strains between patients and parents, it should be kept in mind that there may be changes in cagPAI in the future. In addition, children can be infected with more than one strain of H. pylori at the same time. By increasing the number of biopsy samples, the possibility of detecting other strains of *H. pylori* can be increased.

Since the PCR-RFLP method, which examines the 1162 bp specific region of the *vacA* gene in our study to detect point mutations effective in clarithromycin resistance, is a sensitive method that gives a very heterogeneous band profile, as shown in other studies, it has been concluded that *H. pylori* isolation in gastric biopsy samples taken from pediatric patients is not a suitable method for determining kinship transmission (42).

CONCLUSION

In conclusion, *H. pylori* infection was positive in approximately one-third of the children with dyspeptic complaints. It was observed that these patients mostly had the vacAs2 genotype. Even individuals with at least one of the genetic mutations A2142G and A2143G genetic mutations have the potential to be resistant to antibiotics. A high rate of resistance has been found against clarithromycin used in the standard triple therapy for *H. pylori* in children in our country. *H. pylori* infection eradication rates are also low with standard triple therapy in clinical studies. Therefore, it is appropriate to develop new treatment modalities for treating *H. pylori* infection in children.

Ethics

Ethics Committee Approval: Ethics Committee approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Çukurova University Faculty of Medicine (approval number: 37, date: 05.12.2014).

Informed Consent: The relatives of all the children included in the study were interviewed. After providing information about the interventional procedures to be applied to patients, the purpose of the research, and where to use the data, verbal and written consent was obtained.

Authorship Contributions

Surgical and Medical Practices: N.U.Ü., A.B., M.A., O.Ü., F.D., G.T., Concept: N.U.Ü., A.B., G.T., Design: N.U.Ü., G.T., Data Collection or Processing: N.U.Ü., A.B., T.N., M.A., T.K., O.Ü., F.D., F.K., G.T., Analysis or Interpretation: N.U.Ü., A.B., T.N., M.A., T.K., O.Ü., F.D., F.K., G.T., Literature Search: N.U.Ü., A.B., G.T., Writing: N.U.Ü., A.B., G.T.

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

Financial Disclosure: This study was supported by Çukurova University Rectorate Research Fund TTU-2015- 3550 project.

REFERENCES

1. Ryan, Kenneth J. Topley and Wilson's Principles of Bacteriology, Virology and Immunity, vol 1: General Bacteriology and Immunity. JAMA. 1991; 266: 2621.
2. Baltrus DA, Amieva MR, Covacci A, Lowe TM, Merrel DS, Ottemann KM, et al. The complete genome sequence of Helicobacter pylori strain G27. J Bacteriol. 2009; 191: 447-8.
3. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984; 1: 1311-5.
4. van Amsterdam K, van Vliet AH, Kusters JG, van der Ende A. Of microbe and man: determinants of Helicobacter pylori-related diseases. FEMS Microbiol Rev. 2006; 30: 131-56.
5. EUCAST T. European Committee on Antimicrobial Susceptibility Testing, Breakpoint tables for interpretation of MICs and zone diameters. European Society of Clinical Microbiology and Infectious Diseases Basel; 2015.
6. Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med. 2002; 347: 1175-86.
7. Dunn BE. Pathogenic mechanisms of Helicobacter pylori. Gastroenterol Clin North Am. 1993; 22: 43-57.
8. Höcker M, Hohenberger P. Helicobacter pylori virulence factors--one part of a big picture. Lancet. 2003; 362: 1231-3.
9. Ozbey G, Dogan Y, Demiroren K. Prevalence of Helicobacter pylori virulence genotypes among children in Eastern Turkey. World J Gastroenterol. 2013; 19: 6585-9.
10. Karabiber H, Selimoglu MA, Otlu B, Yildirim O, Ozer A. Virulence factors and antibiotic resistance in children with Helicobacter pylori gastritis. J Pediatr Gastroenterol Nutr. 2014; 58: 608-12.
11. Braga LL, Oliveira MA, Gonçalves MH, Chaves FK, Benigno TGS, Gomes AD, et al. CagA phosphorylation EPIYA-C motifs and the vacA i genotype in Helicobacter pylori strains of asymptomatic children from a high-risk gastric cancer area in northeastern Brazil. Mem Inst Oswaldo Cruz. 2014; 109: 1045-9.
12. Ortiz-Princz D, Daoud G, Salgado-Sabel A, Cavazza ME. Helicobacter pylori infection in children: should it be carefully assessed? Eur Rev Med Pharmacol Sci. 2016; 20: 1798-813.
13. Erdoğdu C, Saribaş Z, Akyön Yilmaz Y. Detection of cagA and vacA genotypes of Helicobacter pylori isolates from a university hospital in Ankara region, Turkey. Turk J Med Sci. 2014; 44: 126-32.
14. Rafeey M, Ghotaslou R, Milani M, Farokhi N, Ghojazadeh M. Association between Helicobacter pylori, cagA, and vacA Status and Clinical Presentation in Iranian Children. Iran J Pediatr. 2013; 23: 551-6.
15. Camorlinga-Ponce M, Torres J, Perez-Perez G, Leal-Herrea Y, Gonzalez-Ortiz B, Madrazp de la Garza A, et al. Validation of a serologic test for the diagnosis of Helicobacter pylori infection and the immune response to urease and CagA in children. Am J Gastroenterol. 1998; 93: 1264-70.
16. Elitsur Y, Neace C, Werthammer MC, Triest WE. Prevalence of CagA, VacA antibodies in symptomatic and asymptomatic children with Helicobacter pylori infection. Helicobacter. 1999; 4: 100-5.
17. Rutigliano V, Ierardi E, Francavilla R, Castellaneta S, Margiotta M, Amoruso A, et al. Helicobacter pylori and nonulcer dyspepsia in childhood: clinical pattern, diagnostic techniques, and bacterial strains. J Pediatr Gastroenterol Nutr. 1999; 28: 296-300.

18. Kato S, Sugiyama T, Kudo M, Ohnuma K, Ozawa K, Iinuma K, et al. CagA antibodies in Japanese children with nodular gastritis or peptic ulcer disease. J Clin Microbiol. 2000; 38: 68-70.
19. Saltik IN, Demir H, Engin D, Ertunç OD, Akyön Y, Koçak N. The cagA status of Helicobacter pylori isolates from dyspeptic children in Turkey. FEMS Immunol Med Microbiol. 2003; 36: 147-9.
20. Sharara AI, Chedid M, Araj GF, Barada KA, Mourad FH. Prevalence of Helicobacter pylori resistance to metronidazole, clarithromycin, amoxycillin and tetracycline in Lebanon. Int J Antimicrob Agents. 2002; 19: 155-8.
21. Lang L, García F. Comparison of E-test and disk diffusion assay to evaluate resistance of Helicobacter pylori isolates to amoxicillin, clarithromycin, metronidazole and tetracycline in Costa Rica. Int J Antimicrob Agents. 2004; 24: 572-7.
22. Alarcón T, Domingo D, Sánchez I, Díaz de Rojas F, López-Brea M. In vitro activity of omeprazole in combination with several antimicrobial agents against clinical isolates of Helicobacter pylori. Eur J Clin Microbiol Infect Dis. 1996; 15: 937-40.
23. Bakir Ozbey S, Ozakin C, Keskin M. Antibiotic resistance rates of Helicobacter pylori isolates and the comparison of E-test and fluorescent in situ hybridization methods for the detection of clarithromycin resistant strains. Mikrobiyol Bul. 2009; 43: 227-34 (Turkish).
24. Onder G, Aydin A, Akarca U, Tekin F, Ozutemiz O, Ilter T. High Helicobacter pylori resistance rate to clarithromycin in Turkey. J Clin Gastroenterol. 2007; 41: 747-50.
25. Sezgin O, Aslan G, Altintaş E, Tezcan S, Serin MS, Emekdaş G. Detection of point mutations on 23S rRNA of Helicobacter pylori and resistance to clarithromycin with PCR-RFLP in gastric biopsy specimens in Mersin, Turkey. Turk J Gastroenterol. 2008; 19: 163-7.
26. Kalach N, Serhal L, Asmar E, Campeotto F, Bergeret M, Dehecq E, et al. Helicobacter pylori primary resistant strains over 11 years in French children. Diagn Microbiol Infect Dis. 2007; 59: 217-22.
27. Vécsei A, Kipet A, Innerhofer A, Graf U, Binder C, Gizci H, et al. Time trends of Helicobacter pylori resistance to antibiotics in children living in Vienna, Austria. Helicobacter. 2010; 15: 214-220.
28. Garcia GT, Aranda KR, Gonçalves ME, Graf U, Binder C, Gizci H, et al. High prevalence of clarithromycin resistance and cagA, vacA, iceA2, and babA2 genotypes of Helicobacter pylori in Brazilian children. J Clin Microbiol. 2010; 48: 4266-8.
29. Ozçay F, Koçak N, Temizel IN, Demir H, Ozen H, Yüce A, et al. Helicobacter pylori infection in Turkish children: comparison of diagnostic tests, evaluation of eradication rate, and changes in symptoms after eradication. Helicobacter. 2004; 9: 242-8.
30. Agudo S, Pérez-Pérez G, Alarcón T, López-Brea M. High prevalence of clarithromycin-resistant Helicobacter pylori strains and risk factors associated with resistance in Madrid, Spain. J Clin Microbiol. 2010; 48: 3703-7.
31. Li L, Ke Y, Yu C, Li G, Yang N, Zhang J, Li Y. Antibiotic resistance of Helicobacter pylori in Chinese children: A multicenter retrospective study over 7 years. Helicobacter. 2017; 22.
32. Okuda M, Kikuchi S, Mabe K, Osaki T, Kamiya S, Fukuda Y, et al. Nationwide survey of Helicobacter pylori treatment for children and adolescents in Japan. Pediatr Int. 2017; 59: 57-61.
33. Wong W, Gu Q, CHU KM, Yee YK, Fung FMY, Tong TSM, et al. Lansoprazole, levofloxacin and amoxicillin triple therapy vs. quadruple therapy as second-line treatment of resistant Helicobacter pylori infection. Aliment Pharmacol Ther. 2006; 23: 421-7.
34. Perna F, Zullo A, Ricci C, Hassan C, Morini S, Vaira D. Levofloxacin-based triple therapy for Helicobacter pylori re-treatment: role of bacterial resistance. Dig Liver Dis. 2007; 39: 1001-5.
35. Liu G, Xu X, He L, Ding Z, Gu Y, Zhang J, et al. Primary antibiotic resistance of Helicobacter pylori isolated from Beijing children. Helicobacter. 2011; 16: 356-62.
36. Nista EC, Candelli M, Zocco MA, Creomini F, Ojetti V, Finizo R, et al. Levofloxacin-based triple therapy in first-line treatment for Helicobacter pylori eradication. Am J Gastroenterol. 2006; 101: 1985-90.
37. Cheng H, Hu FL, Zhang GX, Shi RH, Du YQ, Li ZS, et al. [Levofloxacin-based triple therapy for first-line Helicobacter pylori eradication treatment: a multi-central, randomized, controlled clinical study]. Zhonghua Yi Xue Za Zhi. 2010; 90: 79-82.
38. Cuadrado-Lavín A, Salcines-Caviedes JR, Carrascosa MF, Mellado P, Monteagudo I, Llorca J, et al. Antimicrobial susceptibility of Helicobacter pylori to six antibiotics currently used in Spain. J Antimicrob Chemother. 2012; 67: 170-3.
39. Elviss NC, Owen RJ, Xerry J, Walker AM, Davies K. Helicobacter pylori antibiotic resistance patterns and genotypes in adult dyspeptic patients from a regional population in North Wales. J Antimicrob Chemother. 2004; 54: 435-40.
40. Boyanova L, Nikolov R, Gergova G, Evstatiev I, Lazarova E, Kamburov V, et al. Two-decade trends in primary Helicobacter pylori resistance to antibiotics in Bulgaria. Diagn Microbiol Infect Dis. 2010; 67: 319-26.
41. Caliskan R, Tokman HB, Erzin Y, Saribas S, Yuksel P, Bolek BK, et al. Antimicrobial resistance of Helicobacter pylori strains to five antibiotics, including levofloxacin, in Northwestern Turkey. Rev Soc Bras Med Trop. 2015; 48: 278-84.
42. Han SR, Schreiber HJ, Bhakdi S, Loos M, Maeurer MJ. vacA genotypes and genetic diversity in clinical isolates of Helicobacter pylori. Clin Diagn Lab Immunol. 1998; 5: 139-45.