http:// ijp.mums.ac.ir
Original Article (Pages: 8685-8696)

The Sensitivity of *H. Pylori* in Gastric Tissue Samples of Children and Adolescents to Various Antibiotics in Center of Iran

Fatemeh Famouri[1,2], *Monir Sadat Emadoleslami[2,3], Roya Riahi[4], Hossein Saneian[2,5], Peyman Nasri[1,2]

[1]Assistant Professor, Pediatric Gastroenterology and Hepatology, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non- communicable Disease, Isfahan University of Medical Sciences. [2]Department of Pediatrics, Children Imam-Hossein Hospital, Isfahan University of Medical Sciences, Isfahan, Iran. [3]Assistant Professor of Pediatric Infectious Disease, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non- communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran. [4]MSc of Biostatistics, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non- communicable disease, Isfahan University of Medical Sciences, Isfahan, Iran. [5]Associate Professor of Pediatric Gastroenterology and Hepatology, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non- communicable Disease, Department of Pediatrics, Isfahan University of Medical Sciences, Isfahan, Iran.

Abstract

Background: *Helicobacter pylori (H. pylori)*, is the major known infectious cause of gastric diseases in children and adults. The rate of antibiotic resistance to *H. pylori* treatment regimens has rapidly increased. We aimed to determine the sensitivity of helicobacter pylori in gastric tissue samples of children and adolescents to various antibiotics in Isfahan, Center of Iran.

Materials and Methods: Data set included children and adolescents, aged 5 to 16 years, who have been referred to Imam Hossein Children Hospital in Isfahan, Iran, over 2015 to 2018 due to dyspepsia symptoms. Endoscopy was advised by Pediatric gastroenterologist. Then, Rapid Urease Test (RUT), and one biopsy specimen from antrum, body, andcardia were given and placed into the transfer medium. If RUT became positive, transfer media was transported to the laboratory and keep under 4^{o}C temperature till adding to culture media (Colombia agar- Germany) under special conditions. After appearance of *H. pylori* on culture media, antibiogram was done. We used Epsilometer test (E-test) to determine *H. pylori* sensitivity and resistance to antibiotics as clarithromycin, amoxicillin, metronidazole, tetracyclin, ciprofloxacin and levofloxacin.

Results: Out of 102 patients, E-test of *H. pylori* was positive in 47.1% (n=48) of patients. The highest susceptibility rate was 89.6 % for tetracycline, and 75% for levofloxacin, respectively. Metronidazole had the lowest susceptibility to H. pylori (14.6%). Also, the sensitivity of amoxicillin was low (43.8%).

Conclusion: In this study resistance to primary antibiotic therapy for *H.pylori* eradication (Amoxicillin, Metronidazole, and Clarithromicin) was relatively high in children and adolescents in Center of Iran. Thus, it seems updated treatment strategies based on susceptibility tests are required.

Key Words: Children, Helicobacter pylori, Microbial sensitivity Test, Tissue culture Techniques.

*Please cite this article as: Famouri F, Emadoleslami MS, Riahi R, Saneian H, Nasri P. The Sensitivity of H. Pylori in Gastric Tissue Samples of Children and Adolescents to Various Antibiotics in Center of Iran. Int J Pediatr 2018; 6(12): 8685-96. DOI: **10.22038/ijp.2018.33647.2974**

Corresponding Author:

Monir Sadat Emadoleslami (M.D), Address: 1. Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non- Communicable Disease; Isfahan University of Medical Sciences, Isfahan, Iran. 2. Department of Pediatrics, Children Imam-Hossein Hospital, Isfahan. Postal Code: 8195163381.

Email: emadoleslami@med.mui.ac.ir

Received date: Mar.20, 2018; Accepted date: Jul. 12, 2018

1- INTRODUCTION

Helicobacter pylori (*H. pylori*), a Gram-negative bacteria is the major known cause of gastrointestinal diseases in children and adults. (1). The prevalence of *H. pylori* infection in children is 7.4 to 65% around the world (more than 80% in developing countries and about 40% in the United States) (2). *H. pylori* frequently infect children during the first 5 years of age (3, 4). *H. pylori* infection of the stomach leads to chronic inflammation, atrophic gastritis, peptic ulcer and mucosa-associated lymphoid tissue lymphoma in children and adults. In addition, the infected person can exhibit extra-gastric complications, such as iron deficiency anemia (5). Eradication of *H. pylori* is indicated in a child with *H. pylori* infection and peptic ulcer disease and also in infected children with a history of gastric cancer in first-degree relatives (3).

Eradication therapy of symptomatic *H. pylori* infection considerably prevents the risk of developing gastro duodenal-associated diseases. Recommended therapies are triple and quadruple therapy regimen, composed of two antimicrobial agents (e.g. amoxicillin, metronidazole, tetracycline, and clarithromycin) along with a proton pump inhibitor (PPI), has been widely used to eradicate the microorganism (2). The current recommended primary treatment for eradication of *H. pylori* in children contains any two antibiotics of amoxicillin, metronidazole, or clarithromycin in combination with a proton-pump inhibitor (PPI) for 7 to 14 days (3). Despite evidence of arthropathy in experimental animals following ciprofloxacin administration and relative contraindication of this drug in the pediatric population, the use of quinolones in children is increasing in handling some serious infections (3). Quinolones-based therapies have been used in treating children who have not responded to first-line *H. pylori* treatment, particularly, in the case of multidrug-resistant strains (3). The goal of anti—*H pylori* therapy for susceptible strains is 100% eradication for each regimen, but in developing countries, an effective treatment regimen shows lower rates of success and children usually displays rates of 10% lower than adults using the same triple therapy (4). The resistance of *H. pylori* to antibiotics is the major cause of treatment failure (4). For efficacious elimination of bacteria, it is important to identify the current antibiotic susceptibility profiles of *H. pylori* for updating eradication regimens. Data about antibiotic resistance rates of *H. pylori* in children are few because endoscopy on children is less frequently performed than in adults (3). The efficacy of *H. pylori* treatment varies according to the geographical area (4).The rate of resistance to different antibiotics changes with time and geographic region (3). During recent years, the rate of antibiotic resistance (especially to clarithromycin) has rapidly increased in most countries worldwide (2).

In developing countries, the resistance rate is higher because of an increased use of these drugs. Thus, the efficacious antimicrobial should be determined based on the resistance pattern for successful elimination of this bacteria (4). Therefore, the growing development of resistant strains shows the need to detect primary and secondary resistance rates in each geographic region by performing sensitivity tests. Increasing *H. pylori* resistance to previously effective antibiotics is a great concern. The European multicenter study from 32 centers in 18 European countries indicated a steady rise in clarithromycin resistance and a rapid development of levofloxacin resistance in *H. pylori* strains. Among adults, significant resistance of *H. pylori* were reported for clarithromycin, levofloxacin metronidazole, whereas in children the rate of clarithromycin

resistance was higher (1). Studies from other developing and even developed countries have obviously shown an increasing resistance of *H. pylori,* with high clarithromycin resistance in Japan, Italy, China and Turkey (1). The prevalence of bacterial antibiotic resistance is variable in different regions and seems to be significantly increased with time in many countries. Thus, it is critical to periodically evaluate the *H. pylori* resistance rates to the most commonly used antimicrobial agents for identifying the most effective eradication regimens. The aim of this study was to evaluate directly the sensitivity of the isolated microorganism from gastric tissue to some recommended antibiotics in children and adolescents in Center of Iran.

2- MATERIALS AND METHODS

2-1. Data source

Dataset included children and adolescents between 5 to 16 years old who had been referred to tertiary acute care facility (Imam Hossein Children Hospital) over 2015 to 2018 due to abdominal pain and dyspepsia symptoms who had positive rapid urea test for evaluating *H. pylori* culture and antibiotic sensitivity test. Institutional board approval was acquired.

2-2. Study design

This is a cross-sectional analytical study on children and adolescent patients with abdominal pain and dyspepsia symptoms who referred to academic pediatric gastroenterologist and candidate for endoscopy. The sample size was determined 60 patients according to the prevalence of *H. pylori* in Iranian children (6). For this purpose, 102 patients have undergone endoscopy. Eligible patients would a candidate for upper endoscopy after getting written consent from their parents at Imam Hossein Children Hospital in Isfahan (the Center of Iran). During endoscopy, if gastric erythema, gastric nodularity, duodenal ulceration or erosion were observed, an antral biopsy sample was first taken from the stomach for RUT followed by one biopsy specimen from antrum, body, and cardia and placed into the transfer medium. The transfer media contained thioglycollate, yeast extract, a small amount of agar and Brain Heart Infusion (BHI) media which hoarse blood was added to them. If the urea test was positive, the transfer media would transfer to the laboratory under 4^{o}C temperature till adding to culture media (Colombia agar-Germany) with gaseous atmosphere contains 5% oxygen, 5% carbon dioxide, 2% hydrogen and 88% nitrogen. Then the plates were been kept in the microaerophilic condition in an incubator for a minimum 4 days. *H. pylori* colonies appeared on culture media after 3- 5 days. They were clear, spherical colonies with a diameter of 1- 2 mm.

Then antibiogram was done. There are different methods for antibiogram including disc diffusion agar, Minimal Inhibitory Concentration (MIC), and Epsilometer Test (E- test). Experimental studies show E- test is superior to other methods. So we used E- test in our project and sensitivity and resistance to some more common antibiotics that were recommended for *H.pylori* treatment such as clarithromycin, amoxicillin, metronidazole, and Tetracycline. Ciprofloxacin and levofloxacin were evaluated separately. The results of microbial culture were expressed as MIC and then the sensitivity to each antibiotic would be displayed as a percentage.

2-3. Inclusion and exclusion criteria

Abdominal pain, severe dyspepsia resistant to anti-acid therapies, or gastrointestinal bleeding were requirements to enter our study. People who have been found a persuasive cause of abdominal pain were not included in the study. Those who had a normal endoscopy or negative culture results were excluded.

2-4. Ethic

This study was approved by the Ethics Committee of the Isfahan University of Medical Sciences (ID-number: 293370).

2-5. Statistical analysis

Data were analyzed in SPSS software (version 20.0). At first, the results of tissue cultures for *H. pylori* reported as positive or negative E- tests. Then results of antibiogram for each antibiotic (metronidazole, clarithromycin, amoxicillin, ciprofloxacin, tetracycline, levofloxacin) were separately expressed in form of MIC (mg/ml). Susceptibility rate was calculated for each mentioned antibiotic. P values of 0.05 or less were considered statistically significant. Quantitative data were reported as mean± standard deviation or median (IOR), and quantitative data as a percentage of frequency. Independent T-test and Pearson´s K2 test was applied to examine the relationship between demographic characteristics and *H. pylori* infection. A logistic regression model was used to study the relationship between demographic characteristics and *H. pylori* resistance to each antibiotic under investigation.

3- RESULTS

Out of 102 patients, 51% (n=52) were boys. The mean age of participants was 8.65± 3.88 years. In total, E-test of *H. pylori* were positive in 47.1% of patient (48 individual had positive E-test). The baseline characteristics of patients according to E-test of *H. pylori* are shown in **Table.1**. There was no significant difference between positive and negative groups (P >0.05). The susceptibility rates for all antibiotics and distribution of antibiotics MIC value are shown in **Table.2**.

Table-1: Demographic characteristics of patients according to E-test of *H. pylori.*

Variables	Total	E-test of H. pylori		P-value
		Positive (n = 48)	Negative (n = 54)	
Age	8.65 ± 3.88	8.13 ± 3.92	9.10 ± 3.83	0.21
Weight	27.05 ± 12.61	26.21 ± 13.61	27.82 ±11.70	0.29
BMI	15.71 ± 2.85	15.87 ± 3.23	15.56 ± 2.48	0.94
Gender				0.85
Girl	50 (49%)	24 (50%)	26 (48.1%)	
Boy	52 (51%)	24 (50%)	28 (51.9%)	

H. pylori: Helicobacter pylori; BMI: Body mass index.

Table-2: The susceptibility rates (95% CI) for all antibiotics.

Antibiotics	Susceptibility % (95% CI)	MIC (mg/ml)			MIC (mg/ml)	
		Median (Q25 Q75)	Min	Max	Susceptibility	Resistance
Metronidazole	14.6 % (6.30 25.0)	192 (64 256)	1	256	≤ 4	≥ 8
Amoxicillin	43.8 % (29.2 58.3)	10 (0.06 144)	0.02	256	≤ 1	≥ 2
Levofloxacin	75.0 % (62.5 87.5)	0.44 (0.25 9.13)	0.13	32	≤ 1	≥ 2
Clarithromycin	64.6 % (50.0 77.1)	0.19 (0.13 24)	0.05	256	≤ 1	≥ 2
Tetracycline	89.6 % (81.3 97.9)	0.25 (0.19 0.25)	0.05	256	≤ 2	≥ 4
Ciprofloxacin	64.6 % (50.0 77.1)	0.25 (0.19 16)	0.05	32	≤ 1	≥ 2

MIC: Minimum inhibitory concentration; CI: Confidence interval.

The highest susceptibility rate for tetracycline was (89.6%) with MICs (median) of 0.25 mg/ml and then for levofloxacin (75%) with MICs (median) of 0.38 mg/ml (figure1). Among all antibiotics, Metronidazole had the lowest susceptibility to *H. pylori* (14.6%) with MICs (median) of 2 mg/ml (**Figure.1**). Overall, resistance rate to at least one antibiotic was identified in 2.1% of patients with positive E-test of *H. pylori*, 52.1 % of patients were resistance to two antibiotics, 41.7% were resistance to three antibiotics and 4.2% were resistance to four antibiotics. The maximum resistance rate was observed for the combination of metronidazole, amoxicillin and ciprofloxacin (8 patients [16.6%]), then for a combination of metronidazole and amoxicillin (6 patients [12.5%]) (**Figures 2 and 3**).

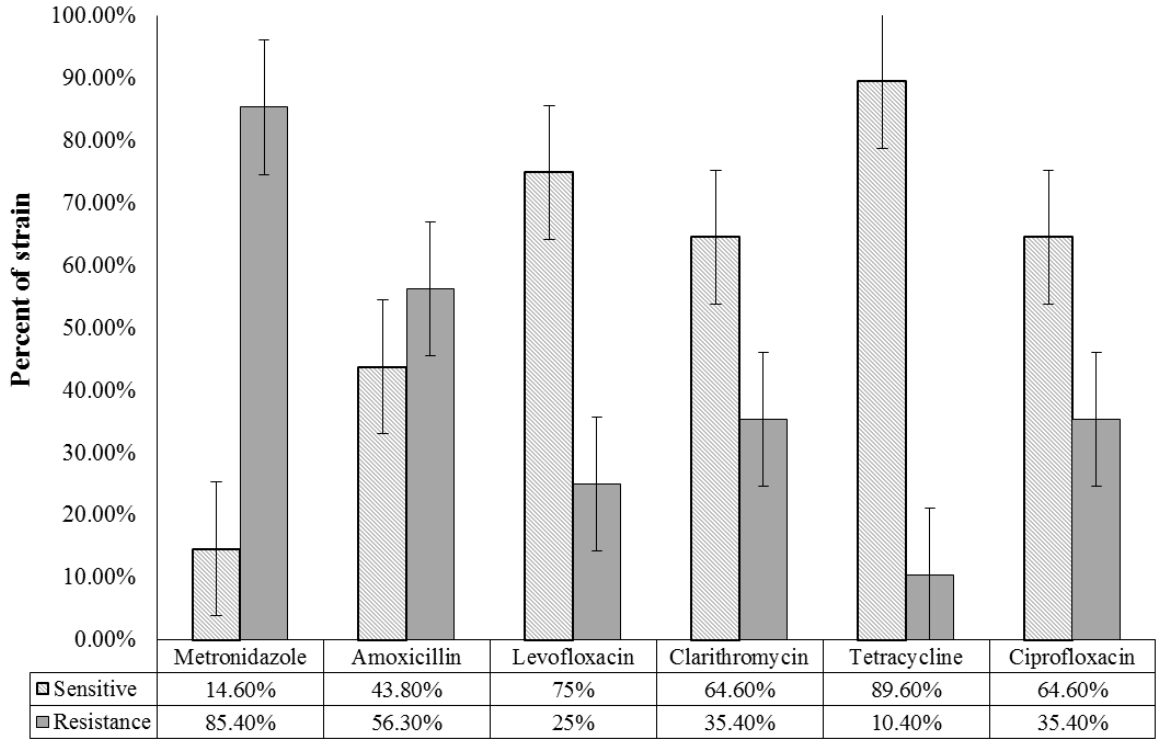
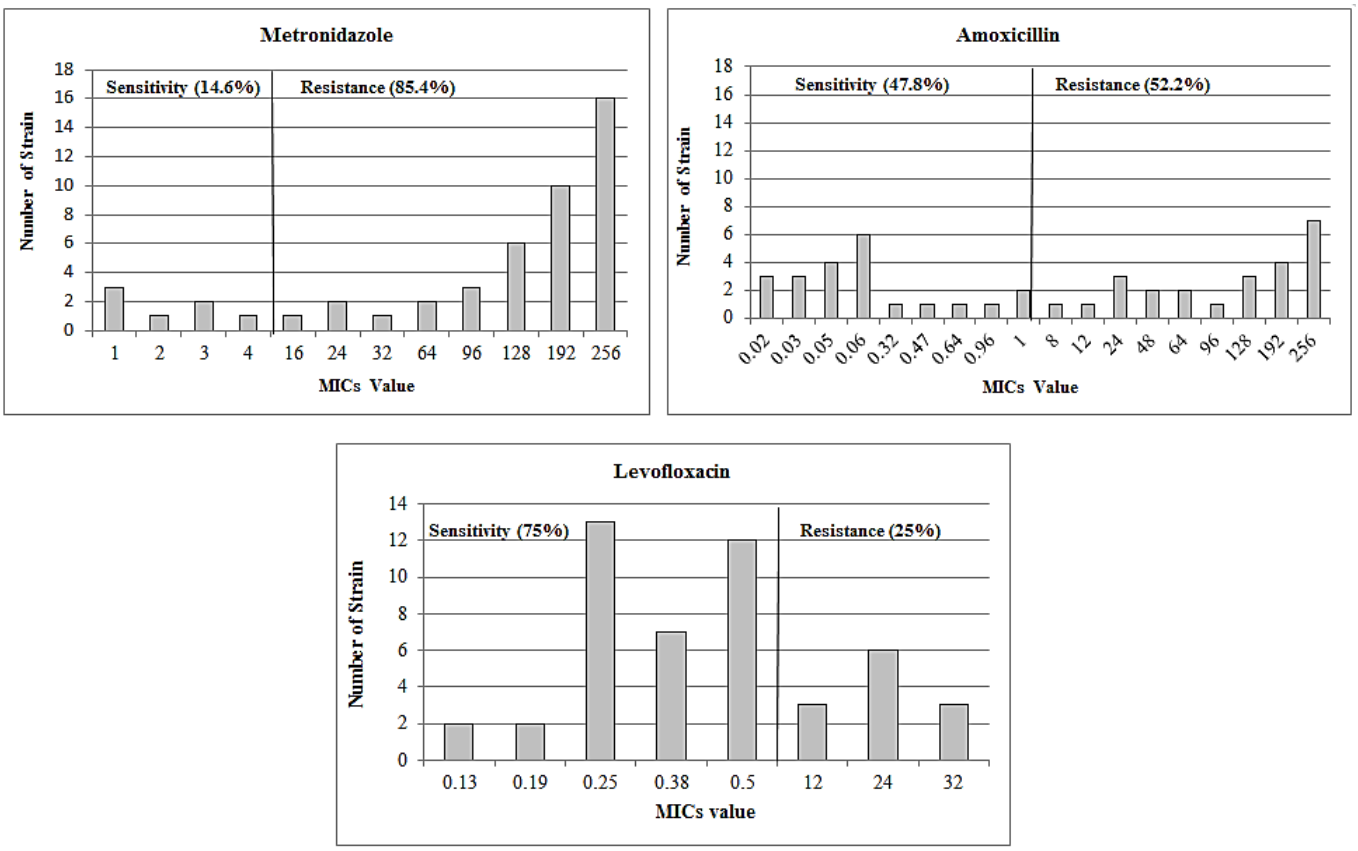
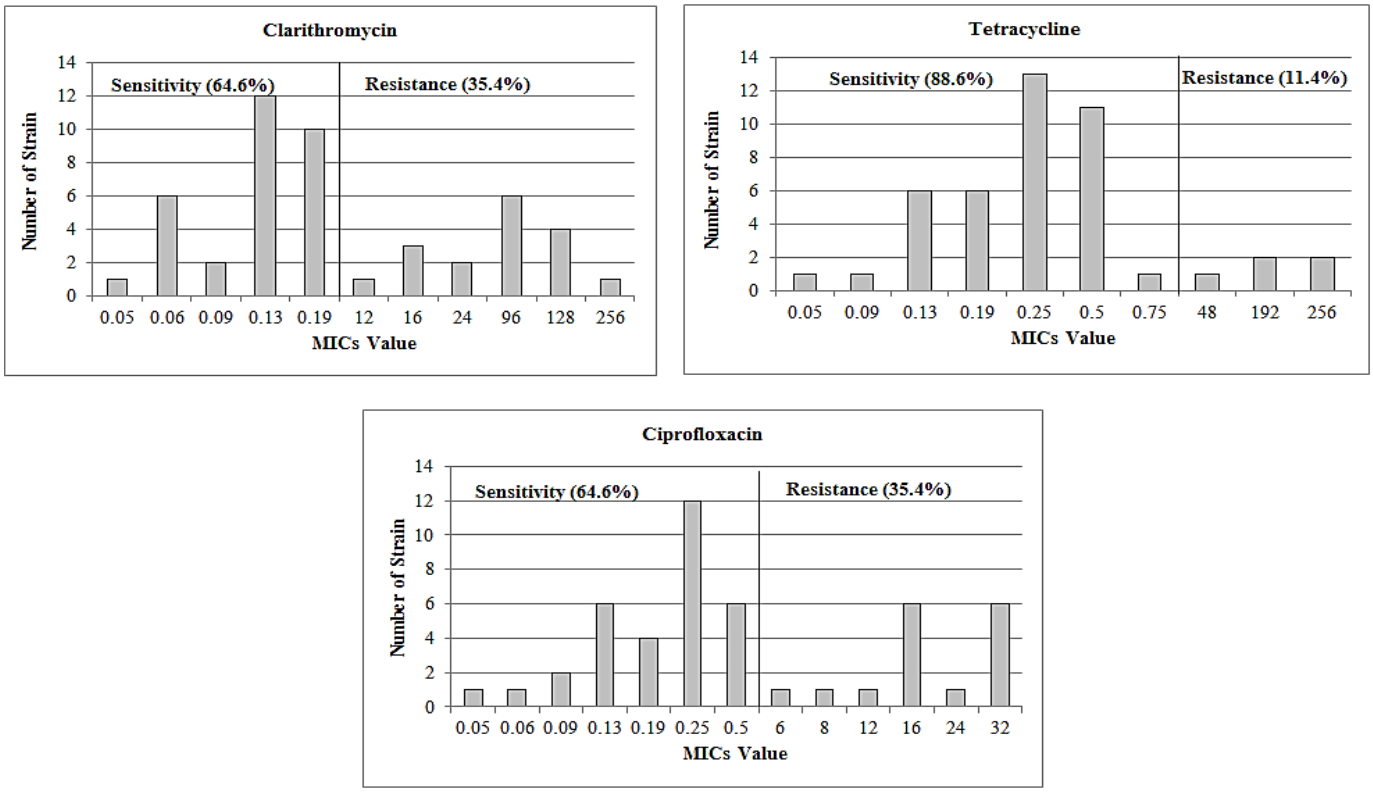


Fig.1: Prevalence of *H. pylori* resistance and susceptibility according to antibiotics type.



MIC: Minimum inhibitory concentration.

Fig.2: Distribution of Metronidazole, Amoxicillin and Levofloxacin MIC value.



MIC: Minimum Inhibitory Concentration.

Fig.3: Distribution of Clarithromycin, Tetracycline, and Ciprofloxacin MIC value.

Prevalence of *H. pylori* susceptibility according to age and sex of participants represented in **Table.3**. *H. pylori* susceptibility rate in girl patients (25%) significantly higher than boy patients (4.2%) (P = 0.041) for metronidazole. Antibiotics resistance rate did not differ among age and sex category for other antibiotics (P > 0.05).

Table-3: The prevalence of *H. pylori* susceptibility according to age and sex category.

Antibiotics	*H. pylori* susceptibility	Age Category		P-value	Gender		P-value
		< 10 years	>= 10 years		Girls	Boys	
Metronidazole	Susceptible	5 (16.1%)	2 (13.3%)	0.80	6 (25%)	1 (4.2%)	0.041
	Resistance	26 (83.9%)	13 (86.7%)		18 (75%)	23 (95.8%)	
	Total	31 (100%)	15 (100%)		24 (100%)	24 (100%)	
Amoxicillin	Susceptible	15 (48.4%)	6 (40%)	0.60	8 (33.3%)	13 (54.2%)	0.15
	Resistance	16 (51.6%)	9 (60%)		16 (54.2%)	11 (45.8%)	
	Total	31 (100%)	15 (100%)		24 (100%)	24 (100%)	
Levofloxacin	Susceptible	21 (67.7%)	13 (86.7%)	0.17	18 (75%)	18 (75%)	0.96
	Resistance	10 (32.3%)	2 (13.3%)		6 (25%)	6 (25%)	
	Total	31 (100%)	16 (100%)		24 (100%)	24 (100%)	
Clarithromycin	Susceptible	20 (64.5%)	9 (60%)	0.77	15 (62.5%)	16 (66.7%)	0.76
	Resistance	11 (62.5%)	6 (40%)		9 (37.5%)	8 (33.3%)	
	Total	31 (100%)	16 (100%)		24 (100%)	24 (100%)	
Tetracycline	Susceptible	27 (87.1%)	14 (93.3%)	0.52	22 (91.7%)	21 (87.5%)	0.64
	Resistance	4 (12.9%)	1 (6.7%)		2 (8.3%)	3 (12.5%)	
	Total	31 (100%)	16 (100%)		24 (100%)	24 (100%)	
Ciprofloxacin	Susceptible	21 (67.7%)	9 (60%)	0.72	16 (66.7%)	15 (62.5%)	0.76
	Resistance	10 (32.3%)	6 (40%)		8 (33.3%)	9 (37.5%)	
	Total	31 (100%)	16 (100%)		23 (100%)	24 (100%)	

H. pylori: Helicobacter pylori.

Table.4 shows, the association between demographic characteristics and *H. pylori* resistance to antibiotics by multiple binary logistic regression. After adjusted model for sex, age and BMI, there was no significant association between age, sex and BMI of participants with odds of *H. pylori* resistance for each antibiotic (P > 0.05).

Table-4: The Relationship of demographic characteristics with *H. pylori* resistance to antibiotics.

Antibiotics	Variables	OR (Resistance/Susceptibility)	95% CI for OR		P-value
			Lower	Upper	
Metronidazole					
	BMI (kg/m^2)	1.20	0.81	1.79	0.37
	Gender (Boys/Girls)	8.17	0.88	75.8	0.06
	Age (≥ 10 years/<10 years)	0.55	0.04	6.73	0.64
Ciprofloxacin					
	BMI (kg/m^2)	1.00	0.80	1.26	0.97
	Gender (Boys/Girls)	1.28	0.37	4.37	0.69
	Age (≥ 10 years/<10 years)	1.38	0.29	6.48	0.70
Tetracycline					
	BMI (kg/m^2)	1.23	0.89	1.69	0.21
	Gender (Boys/Girls)	1.18	0.16	8.47	0.87
	Age (≥ 10 years/<10 years)	0.21	0.01	3.20	0.26
Clarithromycin					
	BMI (kg/m^2)	1.13	0.90	1.42	0.30
	Gender (Boys/Girls)	0.76	0.16	3.68	0.52
	Age (≥ 10 years/<10 years)	0.67	0.20	2.28	0.73
Levofloxacin					
	BMI (kg/m^2)	0.80	0.57	1.12	0.20
	Gender (Boys/Girls)	1.06	0.26	4.27	0.72
	Age (≥ 10 years/<10 years)	0.69	0.09	5.06	0.94
Amoxicillin					
	BMI (kg/m^2)	0.94	0.75	1.19	0.61
	Gender (Boys/Girls)	0.49	0.15	1.63	0.25
	Age (≥ 10 years/<10 years)	1.81	0.38	8.66	0.46

H. pylori: Helicobacter pylori; BMI: Body mass index; CI: confidence interval; OR: Odds ratio.

4- DISCUSSION

Prevalence of *H. pylori* infection is rising worldwide especially in underdeveloped and developing countries (6). We aimed to determine the sensitivity of helicobacter pylori in gastric tissue samples of children and adolescents to various antibiotics in Isfahan, Iran. It showed rising resistance to Amoxicillin, Metronidazole and Clarithromycin. Considering many complications of H. pylori infection in the gastrointestinal tract, antibiotic resistance can create much trouble for community health. The present study was conducted on children and adolescents with symptoms of acid-peptic disease who had been undergone upper gastrointestinal endoscopy. Their RUT

was variably positive. Among 102 patients with a positive RUT that *H. pylori* culture of gastric tissue was directed, 48 individuals had positive E- test (47.1%). This is comparable with the study that directed in Lithuania Lithuania in 2015 on *H. pylori* adults and children (1). *H. pylori* culture with E test method was positive in 28% of adults and 21% of children. Using different transfer media might cause this difference. In addition, we provided one biopsy specimen from each part of the stomach including cardia, body, and antrum due to the possibility of *H. pylori* migration (one additional sample in comparison). Despite positive urea breath test (UBT), only less than half (47.1%) of individuals were culture positive.

This could be due to the specific condition of *H. pylori* growth (7). Also, they are very susceptible, and some of them may die during transferring to the culture media (7). After adjusted model for sex, age, and BMI, there was no significant association between age, gender, and BMI of participants with odds of *H. pylori* resistance for each antibiotic ($P > 0.05$). Triple and quadruple therapy for the first time was advised for primary and secondary treatment of *H. pylori* infection. On the other hand, numerous non-responsiveness to these therapies are reported during the time. Maleknejad et al. (8) conducted a study on primary antibiotic resistance to *H. pylori* strains isolated from children in northern Iran in 2014, using disk diffusion method on Hinton agar media and found that resistance to metronidazole was at the highest rate (57.1%), the lowest rate was for clarithromycin (4.8%), while the resistance rate of amoxicillin was 14.3%. These results were nearly similar to other districts of Iran and neighborhood countries (8). The highest rate of clarithromycin resistance related to Tomatari et al. (9) on adults that was 23% that period of times. The result of our study compared with the previous study in Iran, (35.4% versus 4.8%) revealed that clarithromycin resistance of *H. pylori* has increased in recent years and this could be a tragedy. In one research 2014 Vietnam on children and adults, they found that resistance of *H. pylori* to clarithromycin was 84.6% and 87.7% subsequently (10). Sequence analysis showed point mutation at 23SrRNA in both clarithromycin resistant and sensitive individuals. There was also a T2182C mutation in both of them. They considered this as the most important factor in the lack of appropriate response to treatment in Vietnam and Japan (10). According to an investigation in 2016 USA Myoclinic, the resistance rate of *H. pylori* to clarithromycin was high (70.4%) in contrast to the results of 1993 which showed the least one (6.1%). They also suggested that developing point mutation in the peptidyltransferase region of domain V of the 23SrRNA is the source of this event (10).

The resistance of *H. pylori* to clarithromycin in Lithuania was reported as 8.2% of adults and 17.7% in children in 2015 with E- test accompanied with Real Time PCR (RT-PCR) method. Low prevalence of *H. pylori* infection was reported in this area (1). In this study and another multicenter study which were conducted in Europe, they founded that prevalence of clarithromycin resistance was higher in children than in adults. The lowest rate belonged to Croatia (11.9%) and the highest for Spain (34.7%). Koletzko et al. (11) stated an overall resistance to clarithromycin of 24% (primary resistance in 20% and secondary in 42%) in multicenter studies (11). Resistance to clarithromycin in children in Brazil, Nigeria, Slovenia, Bulgaria were 19.5%, 14.4%, 23.4% and 28.1%, respectively in recent years (4, 5, 12, 13). The result of clarithromycin resistance of *H. pylori* was 35.4% in our center, comparable to the highest rate of Europe.

According to the Maastricht V/Florence Consensus, for considering *H. pylori* eradication triple therapy containing clarithromycin, susceptibility testing should be instructed (14). With increasing *H. pylori* prevalence and necessarily increasing treatment for it, in recent- year resistance to particular antibiotics is increasing so that in our study resistance rate to clarithromycin has risen to 35.4%. The other reason for decreasing clarithromycin sensitivity is growing used of macrolides for respiratory infections. Another cause might be the relative resistance of *H. pylori* to metronidazole and not being able to use tetracycline in many children in triple therapy for *H. pylori* eradication, cause prescribing clarithromycin too much more.

4-1. Metronidazole

Microbial resistance to metronidazole was extremely high (85%) in the present study (**Table.3**); while it was 57.1% in Maleknejad research in 2014 (8). This indicates a rising rate of resistance to this medication. According to the study, it seems that this drug cannot have an effective role in the treatment of *H. pylori* since then. The rate of resistance to metronidazole also reported high in parallel surveys through the recent years. So that it was 99.1% in Nigeria, 40% in Brazil, 33.8% Bulgaria, 25% in Lithuania, 20.2% in Slovenia (1, 4, 5, 12, 13). Resistance rate in some neighborhood countries including Saudi Arabia, India and Pakistan were 80%, 80%, 84%, respectively in 2014 (15). By comparing the results, we find that the resistance rate to this medication in our country is very similar to Nigeria, India and some adjacent nations, but is significantly much higher than some European countries. The rationale for this phenomenon might be the widespread use of this antibiotic not only for parasitic infections, diarrhea disease, and other gastrointestinal diseases but also for other infectious or even surgical problems in our country and Nigeria. Furthermore, metronidazole has been used as a part of the first line treatment protocol of *H. pylori* for many years.

4-2. Amoxicillin

Despite the very low resistance rate to amoxicillin in 2014 (14.3% Iran, it has reached to 66.2% in our investigation (8). This rising can be due to increasing prescription of it for respiratory disease, otitis media, pharyngitis and some other infections. This antibiotic is easily available for patients and is overused for mild upper respiratory infection even without the physician`s permission. Moreover, due to the high prevalence of *H. pylori* in Iran as a developing country, this drug is overused to eradicate it. Compared with neighborhood countries (15), Saudi Arabia (1%), Bangladesh (0%), India (33%) and Pakistan (37%), unfortunately, resistance rate is significantly high in our center. The results of other researches on children and adolescents are as follow: Brazil (10.4%), Slovenia (1%), Bulgaria (4%), Nigeria (33.3%) (4, 5, 12, 13). Resistance to amoxicillin in our center was the highest among all these investigations.

4-3. Tetracycline

The least resistance belonged to tetracycline (10.4%) which has had the lowest rate of prescription for children due to adverse effects in this age range. However, even this amount of resistance would not be expected in children. This represented that resistant species were received from older family members. Reported resistance to tetracycline in other studies is: Nigeria (4.5%), Lithuania (0%), Brazil (0%), Slovenia (0%) (1, 4, 5, 12). Again we have had higher resistance to tetracyclin in relation to other similar scenarios. This is a warning to improve hygiene. Due to low respond to treatment in *H. pylori* positive patients, we surveyed 2 more antibiotics (levofloxacin and ciprofloxacin). Results represented 35.4%

resistance to ciprofloxacin and 25% to levofloxacin. Because of less prescription of these drugs in children in addition to inadequate hygiene in our region, these results are disappointing. Furthermore, growing usage of these antibiotics for the treatment of severe infections resistant to other antibiotics may be another reason. Resistance to levofloxacin compared with our results in Bulgaria and Slovenia were 19.4% and 2.8%, respectively (5, 13).

Overall, the resistance rate to at least one antibiotic was identified in 2.1% of patients with positive E-test of *H. pylori*. Multidrug resistance rate that was so, 52.1% of patients were resistance to two antibiotics, 41.7% were resistance to three antibiotics and 4.2% were resistance to four antibiotics, respectively. This study and other similar investigations indicate that antimicrobials which contributed triple and quadruple therapy may be no longer universal and appropriate treatment for *H. pylori* in children. *H. pylori* infection causes some detrimental health effects such as peptic ulceration, atrophic gastritis, and gastric cancer. Its eradication in some individuals is essential.

Nonetheless, the prevalence of *H. pylori* infection is high especially in developing and underdeveloped countries where estimated in some area up to 90% of adults. Despite increasing antibiotic resistance, total eradication of *H. pylori* seems impossible in humans. By treating every *H. pylori* positive person, eradication cannot be fulfilled in high-risk patients. However, the latest ESPGHAN and NASPGHAN guidelines recommended eradication therapy for *H. pylori* according to the results of antimicrobial susceptibility testing for developed countries (16). Nonetheless, this study provides useful and new information about the antibiotic sensitivity of *H. pylori* Yearly regional *H. pylori* susceptibility testing may help us select an effective eradication protocol.

4-1. Limitations of the study

Limitation of this study includes the inability to carry out PCR and genetic testing in antibiotic-resistant patients.

5- CONCLUSION

Antibiotic resistance to primary *H. pylori* eradication protocol is increasing worldwide especially in developing countries. In this study low susceptibility of *H. pylori* to the primary treatment, the protocol was found. The updated treatment strategies based on susceptibility tests are required. Due to the high antibiotic resistance of *H. pylori* in Iran, we need new policies for its limitation. More researches are needed to evaluate antibiotic prescription in our country. In addition to hygienic improvement, logical antibiotic usage in infectious diseases is recommended.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENT

We would like to thank the Erythron Lab and Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, because this report would not have been possible without the valuable contribution (Project number: 293370)

8- REFERENCES

1. Dargiene G, Kupcinskas J, Jonaitis L, Vezbavicius M, Kadusevicius E, Kupcinskiene E, et al. Primary antibiotic resistance of Helicobacter pylori strains among adults and children in a tertiary referral centre in Lithuania. Apmis 2018;126:21-8.

2. Hu Y, Zhu Y, Lu N-H. Novel and effective therapeutic regimens for Helicobacter pylori in an era of increasing antibiotic resistance. Frontiers in cellular and infection microbiology 2017;7:168.

3. Li L, Ke Y, Yu C, Li G, Yang N, Zhang J, et al. Antibiotic resistance of Helicobacter pylori in Chinese children: a

multicenter retrospective study over 7 years. Helicobacter 2017;22:e12373.

4. Ogata SK, Godoy APO, da Silva Patricio FR, Kawakami E. High Helicobacter pylori resistance to metronidazole and clarithromycin in Brazilian children and adolescents. Journal of pediatric gastroenterology and nutrition 2013;56:645-648.

5. Butenko T, Jeverica S, Orel R, Homan M. Antibacterial resistance and the success of tailored triple therapy in Helicobacter pylori strains isolated from Slovenian children. Helicobacter 2017;22:e12400.

6. Namakin K. Prevalence of Helicobacter pylori infection in asymptomatic children in Birjand, Eastern Iran. International Journal of Pediatrics 2014;2:55-63.

7. Nostro A, Cellini L, Bartolomeo SD, Campli ED, Grande R, Cannatelli M, et al. Antibacterial effect of plant extracts against Helicobacter pylori. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 2005;19:198-202.

8. Maleknejad S, Mojtahedi A, Safaei-Asl A, Taghavi Z, Kazemnejad E. Primary antibiotic resistance to Helicobacter pylori strains isolated from children in Northern Iran: a single center study. Iran J Pediatr. 2015;25(6):e2661.

9. Tomatari FH, Mobarez AM, Amini M, Hosseini D, Abadi ATB. Helicobacter pylori resistance to metronidazole and clarithromycin in dyspeptic patients in Iran. Iranian Red Crescent Medical Journal 2010;12:409.

10. Phan TN, Santona A, Tran VH, Tran TNH, Cappuccinelli P, Rubino S, et al. High rate of levofloxacin resistance in a background of clarithromycin-and metronidazole-resistant Helicobacter pylori in Vietnam. International journal of antimicrobial agents 2015; 45: 244-248.

11. Koletzko S, Richy F, Bontems P, Crone J, Kalach N, Monteiro ML, et al. Prospective multicentre study on antibiotic resistance of Helicobacter pylori strains obtained from children living in Europe. Gut 2006;55:1711-16.

12. Harrison U, Fowora MA, Seriki AT, Loell E, Mueller S, Ugo-Ijeh M, et al. Helicobacter pylori strains from a Nigerian cohort show divergent antibiotic resistance rates and a uniform pathogenicity profile. PloS one 2017;12:e0176454.

13. Boyanova L, Gergova G, Evstatiev I, Spassova Z, Kandilarov N, Yaneva P, et al. Helicobacter pylori resistance to six antibiotics by two breakpoint systems and resistance evolution in Bulgaria. Infectious Diseases 2016;48:56-62.

14. Malfertheiner P, Megraud F, O'morain C, Gisbert J, Kuipers E, Axon A, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence consensus report. Gut 2016:gutjnl-2016-312288.

15. Mohammed Rizwan NF, Alvi A. Epidemiology and pattern of antibiotic resistance in Helicobacter pylori: Scenario from Saudi Arabia. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association 2014;20:212.

16. Jones NL, Koletzko S, Goodman K, Bontems P, Cadranel S, Casswall T, et al. Joint ESPGHAN/NASPGHAN guidelines for the management of Helicobacter pylori in children and adolescents (update 2016). J Pediatr Gastroenterol Nutr. 2017; 64(6):991-1003.