International Journal of Molecular and Clinical Microbiology 7(2) (2017) 824-833

International Journal of
Molecular and Clinical Microbiology

# A study on Antimicrobial Resistance of *Helicobacter pylori* Isolates Recovered from Dyspeptic Patients by Epsilometer Test

**Alireza Massiha[1*] and Mohammad Reza Majid Khoshkholgh Pahlaviani[1]**

*1. Department of Biotechnology, Lahijan branch, Islamic Azad University of Iran*

ARTICLE INFO

*Article history:*
Received 18 September 2017
Accepted 27 November 2017
Available online 1 December 2017

*Keywords:*
*Gastroduodenal disorders;*
*Helicobacter pylori;*
*Antibiotic resistant;*
*E-test*

ABSTRACT

The main purpose of the study is to determine resistance pattern of Helicobacter pylori isolates to selected antibiotics among 88 dyspeptic patients in Iran. Frequency of infection among these patients have been evaluated among dyspeptic patients old (45 boys and 43 girls, aged 5-18 years) from 2016 to 2017. Urease test, modified Giemsa stain, culture and histopathology of *H. pylori* isolates were performed as per standard guidelines. *H. pylori* ATCC 43504 was used as reference strain. Susceptibility of *H. pylori* isolates to amoxicillin, tetracycline, metronidazole, clarithromycin and levofloxacin was determined using EUCAST break points by E-test method. The most common findings were antral gastritis 14/88 (15.91%). Furthermore, the correlation of culture with modified Giemsa method showed that the proportion of Giemsa-stain test positivity was higher among those, which are culture positive (P>0.05). The three tests culture, giemsa, and urease test found to be significant statistically (P<0.05). *H. pylori* isolates indicated the most sensitivity against tetracycline, amoxicillin, and levofloxacin. The most rates of resistance of HP isolates were shown to Clarithromycin and metronidazole. Findings showed that the MICs of therapeutic antibiotics for eradication of *H. pylori* have increased in the past 10 years in northern Iran. Culture and histopathology, urease test, modified giemsa smear and antibiogram results proved to be helpful in the diagnosis and treatment of *H. pylori* infection. Antimicrobial susceptibility testing can play an important role in management of drug resistant cases of isolates in our country.

## 1. Introduction

*Helicobacter pylori* (*H. pylori*) infection nowadays is recognized as an important causative organism for peptic ulcer, gastric cancer and chronic atrophic gastritis in humans. *H. pylori* is regarded the most common bacterial infection in the world with an estimated 75% of population in developing country being infected with organism even at early age (Smith et al., 2009; Akçam et al., 2010; Pandeya et al., 2011). Several studies have shown conclusively that HP is declining in Asia. However, studies regarding HP prevalence in different parts of Iran are limited (Hashemi et al., 2006). The results of various studies throughout the world that have examined *H. pylori* resistance rates compared to the main drugs used in antimicrobial therapy varies significantely, confirming the hypothesis that antibacterial resistance is a highly local phenomenon (Biernat et al.,2014). Therefore, it is important to understand the distribution of the

*Corresponding author: Dr. Alireza Massiha
Tel: +98-9111318420
E-mail address: amirmassiha@liau.ac.ir

levels of specific genes associated with antimicrobial resistance in order to ascertain how the characteristics of given population can be influenced which therapy is the most appropriate. For choosing the best treatment regimen, a local susceptibility pattern is necessary. Epidemiologic studies have indicated that ingestion of contaminated food may increase the risk of HP infection. Person-to-person transmission by oral-oral, faecal-oral or gastro-oral exposure is suggested to be the most likely route of transmission (Dorji et al., 2014; Brown, 2000). Consequently, advances in hygiene and living conditions are effective factors in decreasing the prevalence of infection (Vale et al., 2010). Additionally, mammal and insect reservoirs have been proposed such as pigtailed monkeys, rhesus monkeys, cats, sheep and cockroaches (Khalifa et al., 2010). Most observational studies report an association between maternal infection and socioeconomic status being an important risk factor for infant infection (Bassily et al., 1999; Yang et al., 2005; Kivi et al., 2005; Weyermann et al., 2009; Opekun et al., 2009). On this basis, monitoring antibiotic resistance prevalence is meaningful for HP infection management in clinical practice. For this reason, detection of susceptibility in each area for every year can help to make easier way to understand of resistance impact on therapy outcome. Our purpose was to analyze the susceptibility of HP isolates to five usual antibiotics currently used in a northern Iran.

## 2. Materials and Methods

### *2.1. Study Population*

A descriptive cross-sectional research was conducted in pediatric ward in 22 Aban Hospital (Lahijan, Iran) over the period 2016-2017. 88 children and adolescents (45 boys and 43 girls), aged from 5 to 18 years clinically diagnosed as dyspeptic patient underwent upper GI endoscopy accompanied by culture, histology and determination of antibiotic resistance of HP isolates. The patients were divided into three groups, based on their age (A= 5-6) years, B= 7-12 years, C=13-18 years). Oral and written consent was obtained from all participants according to the Declaration of Helsinki. The patients were asked to fill up a questionnaire based on sex, residency and symptom history. At the presentation, the majority of patients complained about upper abdominal pain and burning in the area retrosternal, other symptoms have been reported include nausea, vomiting, and in one case has been hematemesis. Patients were excluded if they had recent use of antibiotics within 1 month, proton pump inhibitors within 2 weeks and histamine receptor antagonists within 1 week prior to endoscopy and those with GI bleeding within the last 7 days. Through endoscopy, five gastric biopsy specimens were gathered as follows: Histology (one from the gastric antrum and the other from the gastric corpus), urease test (one specimen from the angular incisure), culture and antibiogram (one from the gastric antrum and the other from the gastric corpus).

### *2.2. Histology*

Two biopsy specimens each from the corpus and antrum were fixed in neutral 10% buffered formalin overnight and were embedded in paraffin. Two-micrometer-thick sections were stained with Hematoxylin-eosin and one section was stained by the modified Giemsa procedure and examined. The slides were microscopically examined by using high power field (×1000). The presence of curved shaped rod micro-organisms resembling *H. pylori* that stained with Hematoxylin-eosin and modified Giemsa procedures was considered as positive. All examinations were performed under code essentially following the Sydney System recommendations (Dixon et al., 1996).

### *2.3. In-House Urease Test*

Collected biopsy specimens of each patient were incubated in premade broth for the urease test. The test was considered positive when the color of RUT reagent changed from yellow to orange, pink, or purple within four hours of incubation at 25°C (Thillainayagam et al., 1991).

### *2.4. H. pylori culture*

Obtained biopsies were transported to the microbiology laboratory in screw cap bottles with 0.5ml nutrient broth using Stuart's transport medium. The homogenized biopsy specimen was streaked on Blood agar, Chocolate agar and Modified Thayer Martin agar (Hi-Media, India)

augmented with 7% sterile, lysed horse blood and antibiotics supplements (vancomycin 2.0 mg, polymyxin 0.05 mg, trimethoprim 1.0 mg) (Merck, Germany). The plates were incubated under microaerobic conditions at 37˚C for seven days. To achieve relatively high humidity a wet paper towel placed at the bottom of the jar. Growth was observed for at 3, 5 and 7 days. Growth generally appeared by 5 days. If no growth was seen on day 7, the plates were discarded and specimen labeled negative for HP. Isolates were identified by typical colony morphology, Small, translucent to yellowish colonies on lysed blood agar and with translucent to pale grayish colonies on blood agar and tiny colonies, 0.5-1mm, round, low convex, pale, grey-colored, translucent, smooth and easily emulsifiable on Modified Thayer Martin medium were confirmed by hanging drop, catalase, oxidase, rapid urease tests, inability to reduce nitrates, hydrolyse hippurate and resistance to Nalidixic Acid and modified Gram staining in our laboratory (Thillainayagam et al., 1991; Soltesz et al., 1992). Stock cultures stored in Brucella broth with 20% glycerol at -80$^{oC}$ (Ansorg et al., 1991). *H. pylori* strain ATCC 43504 was used as positive control.

### *2.5. Determination of Antibiotic Resistance Patterns by E-test method*

For determining MICs, assessment of HP strains to antimicrobial agents was performed by E-test method, (AB Biodisk, Solna, Sweden). The susceptibility to selected antibiotics (amoxicillin, tetracycline, metronidazole, clarithromycin and levofloxacin) was tested for each *H. pylori* strain. Pure colonies were used to prepare microbial suspension in BHI broth, equivalent to No.3 McFarland standard (approximately $1.0\times10^9$ cfu/mL) (Wang et al., 2001; Toledo et al., 2009). Mueller-Hinton Agar supplemented with 5% sheep blood was used for susceptibility testing. E-test stripes were placed on plates according to manual of the manufacturer separately for selected antibiotics. Petri plates were incubated in microaerobic environment at 37°C for 72 hrs. The determination of MIC values was carried out against the reference HP strain, to ensure the quality of susceptibility tests. *H. pylori* 26695 was used as a negative control. Susceptibility testing was performed for selected antibiotics are summarized in Table 4. Strains classified as susceptible or resistant, according the latest EUCAST breakpoint criteria (Jorgensen et al., 2015).

### *2.6. Statistical Analysis*

Statistical computations were performed by Chi squared and Fisher's exact tests. Analyses were done using the SigmaStat software program. $P < 0.05$ was accepted as statistically significant.

## 3. Results

Biopsy specimens of 88 consecutive patients (45 boys and 43 girls, aged 5-18 years) undergoing endoscopy entered the study. The majority of patients (93%) complained about upper abdominal pain and burning in the area retrosternal, other symptoms have been reported include nausea, vomiting, and in one case has been hematemesis. Abundance of HP among patients with dyspepsia by age and sex among children under 6 years old (4.5%), children 6 to 12 years old (12.5%) and children between 13 and 18 years of age (17.0%) was observed. Overall, the maximum values were related to 13 and 18 years old (Table 1). No statistically significant differences were revealed with the sex and age of patients (P>0.05). Bacteriological results of HP of the studied patients as the majority of the patients were negative to HP culture for 81.8%, while 18.2% of the patients were positive to HP culture (Table 2). Based on the result of the RUT test, majority of the patients were negative to RUT by 67% with only 23.9% of the patients were positive to it. The correlation of modified Giemsa stain and urease test showed that Gram-negative curved bacilli were detected more among the cases, which were urease positive as compared to those which are urease negative (P>0.05). In this study, modified Giemsa procedure was positive in 13.63% (12/88). Furthermore, the correlation of culture with modified Giemsa method showed that the proportion of Giemsa -stain test positivity was higher among those, which are culture positive (P>0.05). Following study, approximately half the patients studied had normal mucosa (n=45). When presence of HP among these groups of patients were compared with histologic results; it was found that from 54

patients with FD, 10 patients showed inactive chronic gastritis and 4 patients showed chronic active gastritis. From 45 cases of normal endoscopy reports only 36 cases of patients demonstrated normal histology and 9 other cases display atrophic changes were observed in histology that associated with the presence of HP. The most common findings were antral gastritis 14/88 (15.91%), followed by duodenal ulcer and peptic ulcer (Tables 2 and 3). Table 4 shows the resulting MIC determination using E-test strips. Totally, with AMX MICs ranging were from 0.016 μg/mL to 0.064 μg/mL in 20 isolates. Because the breakpoint for AMX is 0.12 μg/mL, all of the isolates were deemed to be susceptible to AMX. All isolates of HP were also found to be sensitive to TET. Seven clinical isolates of HP were found to be resistant to MTZ (8%) because their MIC values were ≥64 μg/mL, which is higher than the breakpoint value of 8 μg/mL. Quinolone resistance was observed in six isolates (7%), which had MIC values much greater (>6 μg/mL) than the 1 μg/mL breakpoint. Finally, CLR testing showed that seven isolates had the maximum MIC values that were listed on the E-test strip (256 μg/mL), while one was 128 μg/mL; giving rise to a total of eight resistant isolates (10%) that were well above the 0.5 μg/mL breakpoint. There was at least one antibiotic resistance found in 15 of the isolates (17%).Statistically, resistance rate to selected antibiotics was not significant with the gender of patients (P>0.05). Also, there were no differences in the antimicrobial resistance rates and diseases including GU, DU, gastritis and non-ulcer dyspepsia with different age groups (P>0.05). MIC values (mg/L) for these antibiotics were as follows: ≤0.125 = susceptible and >0.125= resistant for amoxicillin; ≤0.25=susceptible and >0.5=resistant for clarithromycin; ≤1=susceptible and >1= resistant for tetracycline and levofloxacin; ≤8=susceptible and >8=resistant for metronidazole. The breakpoints were interpreted according to EUCAST clinical breakpoints recommendations. (Table 4) (Matuschek et al., 2014; Cavallo et al., 2008; Bogaerts et al., 2006).

**Table 1.** Distribution of dyspeptic patients based on age and year

| Patients (No.) | | |
|---|---|---|
| | Male | Female |
| Gender | 45 | 43 |
| | 51.1% | 48.9% |
| Mean (SD) | 13.25 | 41.61 |
| Age | | |
| < 6 yr | 1 | 3 |
| 6 - 12 yr | 6 | 5 |
| 13 - 18 yr | 9 | 6 |
| Total (%) | 16 (35.56) | 14 (32.56) |

**Table 2.** The microbiology and histopathologic findings of the studied patients

| Bacteriology findings | | No. | % |
|---|---|---|---|
| Urease test | Positive | 21 | 23.9 |
| | Negative | 67 | 76.1 |
| | Total | 88 | 100 |
| Modified Giemsa -stain | Detected | 12 | 13.6 |
| | Undetected | 76 | 86.4 |
| | Total | 88 | 100 |
| Histopathology | Positive | 15 | 17.0 |
| | Negative | 73 | 83.0 |
| | Total | 88 | 100 |
| Culture | positive | 16 | 18.2 |
| | Negative | 72 | 81.8 |
| | Total | 88 | 100 |

Table 3: Clinical features of HP+ patients based on endoscopy and histopathologic findings

| No. | NM | DU | GC | G | PU |
|---|---|---|---|---|---|
| Positive cases | 45 | 8 | 1 | 14 | 6 |
| Negative cases | 43 | 80 | 87 | 74 | 82 |
| Total | 88 | 88 | 88 | 88 | 88 |

NM-Normal Mucosa, DU-Duodenal ulcer, GC-Gastric Cancer, G- Gastritis, PU-Peptic Ulcer

Table 4: Resistance profile of 88 *Helicobacter pylori* isolates according to E-test method

| Antibiotic | Isolates, n (%) | Quality Control (M.I.C. µg/mL) | EUCAST Interpretation M.I.C. Criteria (µg/mL) | |
|---|---|---|---|---|
| | Resistant | *H. pylori* ATCC 43504 | S | R |
| AMX | 0 (0) | 0.015 - 0.12 | ≤ 0.125 | > 0.125 |
| LVX | 6 (7) | 0.015 - 0.12 | ≤ 1 | > 1 |
| CLR | 8 (10) | 0.015 - 0.12 | ≤ 0.25 | > 0.5 |
| MTZ | 7 (8) | 64 - 256 | ≤ 8 | > 8 |
| TET | 0 (0) | 0.12 - 1 | ≤ 1 | > 1 |

AMX Amoxicillin; LVX Levofloxacin; CLR Clarithromycin; MTZ Metronidazole; TET Tetracycline

## 4. Discussion

Histological examination is considered the gold standard for HP diagnosis. It also can provide an accurate diagnosis of gastric pathology (Tolia et al., 2000). There was a significant relationship between age and HP ($P< 0.05$) in our study, whereas others found no significant correlation between HP infection and age (Akbar et al., 2005; Petrović et al., 2010). In this study, a higher incidence of the HP was indicated among those aged 13–18 years. WHO found that the majority of infections occurred in young age groups rather than other age groups and the factors that predispose the higher colonization rates included poor socioeconomic status and less education in addition to genetic factors. The explanations for the present study are in agreement with approved results of other studies which were mainly due to socioeconomic status and the sample size of the population studied, type of patients, location of the study as well as the mode of transmission whereby spread infection was acquired from person to person or by oral-oral or feco-oral routes (Drumm et al., 2003; Shuker et al., 2007). *Helicobacter pylori* infection is one of the most common infections in human. Infection is mainly acquired in early childhood. Prevalence of such infection in pediatric age group is higher in developing countries. Re-infection chance is also more among children of such regions. Infection is transmitted mostly through feco-oral route in countries like ours. Only 15% infected children develop specific clinical conditions. Many questions remain unanswered regarding pathophysiology. But an interaction among host, environment and *H. pylori* has been proposed. These factors contribute whether the child will be asymptomatic or symptomatic. Again, these are related in development of nature of disease conditions. Identification effect from H. pylori infection is ambitious. It remains a mainstream challenge for clinicians and basic workers. However, if it is achieved, will make *H. pylori* related disease preventable through available effective treatment regimen (Dinić et al., 2007; Fayed et al., 2011; WGO Global Guideline, 2010). Other researchers observed a higher prevalence of positive HP infection (71%) among individuals between 35-75 years of age, when compared with frequency in the other age groups (Tolia et al., 2000). Following culture of specimen; HP was observed in 18.18 % of gastric biopsies from 88 patients with upper gastrointestinal symptoms. V various HP detection results in gastric biopsies (54.9%; 65%; 73.98%; 75.4 %,) were previously reported (Iman et al., 2008; Bakka et al., 2003; Ayoola et al., 2004; Mohamed et al., 1994). The prevalence of *H. pylori* infection is strongly correlated with socioeconomic status but there are also wide differences between and within countries (Graham et al., 1991; Vanderplas et al., 2001; Suerbaum et al., 2002; Frenck et al., 2003). Variation in study powers as well as ethnicity, place of birth, socioeconomic factors, diet, occupation, smoking, or alcohol consumption habits among study populations may be the reasons for erratic rates of *H pylori* infection reported from the country. As with most infectious and non-infectious diseases, no one factor can be singled out as the major determinant of *H. pylori* incidence and prevalence. However, there is credible evidence that poverty-associated factors are major players. The studies reviewed in this article show some evident differences in epidemiology between developing and developed countries, notably among children. However, we do not think that H. pylori prevalence is directly correlated with a country's overall wealth inasmuch as human poverty is not necessarily dependent on a country's gross domestic product. Instead, the effects of poverty on *H. pylori* infection is more pronounced between different communities, often located within the same country or region, but separated based on sanitation, overall hygiene, and standards of living.

The prevalence of HP infection was different among countries and within a country (Sasidharan et al., 2008). Investigations in different age groups from different regions of Iran reported that HP infection occurs in 57%-91% of the study subjects (Alborzi et al., 2006; Bafandeh et al., 2004). Regarding endoscopic finding, HP infection was present in 15.91% among gastritis, 9.09 % among duodenitis and 6.82 % in patients with peptic ulcer disease. The frequency of HP infection among those with endoscopic diagnosis of gastritis ranged from 60-80% (Maaroos et al., 1990; Jemilohun et al., 2010; Al-Akwaa et al., 2010). Peptic ulcer frequencies are divergent in reports from different countries. In a literature review by the

American Gastroenterology Association, 19 out of 41 studies report duodenal ulcer in ≥ 10% of dyspeptic patients and the overall prevalence of PU in these groups of symptomatic patients is ≥ 15% in 21 studies (Talley et al., 1998). Duodenal ulcer was approximately the same as common as PU in the present survey, which is in quite contrast to the 12:1 ratio reported from India (Malekzadeh et al., 2004). Moreover, HP infection is significantly more frequent in PU than in non-ulcer dyspepsia (DU) patients. Regarding the significantly higher rate of HP infection in those with PU and DU in comparison with the subjects with normal endoscopic findings, Gastric ulcer was similar to duodenal ulcer in the present survey, which is in quite contrast to the 12:1 ratio reported from India (Malekzadeh et al., 2004). Nevertheless, in another large cohort of residents in northwest of Iran, the frequency of PU is just 4.9% (Bafandeh et al., 2004; Massarrat et al., 1995). In this study, relatively low frequency of PU might be due to the enrollment of unnecessarily dyspeptic subjects in the latter survey. Similar to other studies HP was not significantly more prevalent in males (Malekzadeh et al., 2004; Singh et al., 2002; Perez-Perez et al., 2004; Talley et al., 1998). In this work, the correlation of modified Gram-stain and urease test showed that gram negative curved bacilli were detected more among the cases, which were urease positive as compared to those which are urease negative, a statistically significant result ($P < 0.05$). In this study, urease test is positive in 21/88 (23.86%), which was not consistent with the study of Yakoob et al. which gives a result of 40% (Yakoob et al., 2005). Vijaya and Chadrashekhar give a higher percentage of urease test positive (74/100) accounting to 74%. While Nichols et al. give a low percentage of 18.5% positive results by urease test, Berry and Sagar in their study found only 10.9% positive by urease test. The present study was the first to examine antimicrobial susceptibility in Northern Iran. It is surprising that a relatively high percentage of resistance to certain antibiotics was found by E-test, namely MTZ (8%), CLR (10%) and LVX (7%), in spite of small number of HP isolates in this study. The data advise that not only will physicians need to identify HP quickly, but also that susceptibility testing should be part of the clinical evaluation so that treatment can be tailored to combat the infection more effectively, rather than relying on consensus guidelines, and to prevent or slow the development of antimicrobial resistance. The British Society for Antimicrobial Chemotherapy, EUCAST and the CLSI do not suggest the use of antibiotic disks for clinical studies because the stability of the antibiotics on the disk may be compromised after 48 h to 72 h of growth, which is normally required for testing HP. Regardless, many laboratories still use this approach as a cost-effective measure to monitor emerging resistant HP strains. The agar dilution method is considered to be successful in large batch studies to provide accurate MICs, but is technically too demanding to be a viable test for everyday practice (Osato et al., 2000; DeCross et al., 1993). The E-test method usually has a more stable antibiotic delivering, is able to tolerate prolonged incubation and can provide MICs (Cederbrant et al., 1993). However, an E-test strip costs approximately 100 times more than an antimicrobial disk, which can be prohibitive in terms of cost and access in developing countries where they try to establish surveillance programs, especially because incidence rates of HP are often higher in some developing nations (Graham et al., 1991). As such, laboratories in countries such as Turkey continue to use disk diffusion to determine susceptible and resistant HP strains, while efficiently selecting for more effective therapies (Ozbey et al., 2012). If the cost and the ease of evaluation are prohibitive factors in screening for HP antimicrobial resistance, disk diffusion, with the possible exception of MTZ, should be an acceptable approach through the development of standardized zones of inhibition. Only one isolate was concurrently resistant to Levofloxacin and Clarithromycin. Dharmalingam et al. in their study reported 100% sensitivity to tetracycline, 99.1% sensitive to amoxicillin, 89.1% sensitive to clarithromycin, while 89.1% of the isolates showed resistance to metronidazole (Dharmalingam et al., 2003). Sharma et al. in their study found a 66% resistance to Metronidazole; their study stated that metronidazole being cheaper ad readily available is used for self-medication and prescribed very commonly for GI disorders like amoebiasis. The high frequency of resistance to metronidazole may be due to repeated exposure of HP to this drug (Sharma et al., 1995). The present study

has shown the minimum amount of resistant HP isolates from patients to levofloxacin and increasing number of resistant isolates to clarithromycin. Finally, the eradication using first-line therapy with clarithromycin and amoxicillin is decreased, especially in the clarithromycin-resistant group, and clarithromycin resistance was considered crucial for the eradication of HP. Nevertheless, eradication of HP is greatly dependent on regular monitoring of antimicrobial susceptibility, which is necessary for selection of an appropriate therapeutic regimen. Moreover, multidrug resistance of HP isolates should be invariably monitored due to the discrepancy of the prevalence of resistant HP strains. New promising antibiotics such as clindamycin, erythromycin and others to overcome increasing resistance rate of traditional used antibiotics in eradication of HP. In developing countries, *H pylori* infection is usually acquired during the first year of life. For children with persistent or severe upper abdominal symptoms, upper endoscopy with biopsy should be the investigations of choice, and the C-UBT is recommended as the best non-invasive diagnostic test that has the added advantages of being able rule out non-H pylori related complications. The stool antigen test has also been recommended consensus guidelines for the management of treatment and eradication need to be stablished in the Middle East for young adults and children based on local data and clinical and laboratory practices (Lee et al., 2014; Asaka et al., 2010). Our study and findings support the need of further larger prospective studies which need more time and a higher budget.

## Acknowledgments

We would like to express our appreciation to the directors and staff of Pathology Department of Razi Hospital, and Rasht Central Pathobiology Laboratory for their cooperation in conducting this study.

## References

Akbar, D.H., Eltahawy, A.T., 2005. *Helicobacter pylori* infection at a university hospital in Saudi Arabia: prevalence, comparison of diagnostic modalities and endoscopic findings. Indian J Pathol Microbiol. 48(2):181-5.

Akçam, M., 2010. *H. pylori* and micronutrients. Indian pediatrics. 47(2):119-26.

Al-Akwaa, A.M., 2010. Prevalence of *Helicobacter pylori* infection in a group of morbidly obese Saudi patients undergoing bariatric surgery: a preliminary report. Saudi J Gastroenterol. 16(4):264-7.

Alborzi, A., Soltani, J., Pourabbas, B., Oboodi, B., Haghighat, M., Hayati, M., Rashidi, M., 2006. Prevalence of *Helicobacter pylori* infection in children (south of Iran). Diagn Microbiol Infect Dis. 54(4):259-61.

Ansorg, R., Von Recklinghausen, G., Pomarius, R., Schmid, E.N., 1991. Evaluation of techniques for isolation, subcultivation, and preservation of *Helicobacter pylori*. Journal of clinical microbiology. 29(1):51-3.

Asaka, M., Kato, M., Takahashi, S., Fukuda, Y., Sugiyama, T., Ota, H., Uemura, N., Murakami, K., Satoh, K., Sugano, K., 2010. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. Helicobacter. 15(1):1-20.

Bafandeh, Y., Esmaeeli, H., Aharizad, S., 2005. *Helicobacter pylori* infection rates in duodenal ulcer patients in a population with high prevalence of infection. Indian journal of gastroenterology: official J. Indian Soci. Gastroenterol. 24(3): 130-130.

Bakka, A.S., El-Gariani, A.B., AbouGhrara, F.M., Salih, B.A., 2002. Frequency of *Helicobacter pylori* infection in dyspeptic patients in Libya. Saudi med J. 23(10):1261-5.

Bassily, S., Frenck, R.W., Mohareb, E.W., Wierzba, T., Savarino, S., Hall, E., Kotkat, A., Naficy, A., Hyams, K.C., Clemens, J., 1999. Seroprevalence of *Helicobacter pylori* among Egyptian newborns and their mothers: a preliminary report. AJTMH. 61(1):37-40.

Biernat, M.M., Poniewierka, E., Blaszczuk, J., Czapla, L., Kempinski, R., Ksiadzyna, D., 2014. Antimicrobial susceptibility of *Helicobacter pylori* isolates from Lower Silesia, Poland. Arch Med Sci.10:505-9

Bogaerts, P., Berhin, C., Nizet, H., Glupczynski, Y., 2006. Prevalence and mechanisms of resistance to fluoroquinolones in *Helicobacter pylori* strains from patients living in Belgium. Helicobacter. 11(5):441-5.

Brown, L.M., 2000. *Helicobacter pylori*: epidemiology and routes of transmission. Epidemiol Rev. 22(2):283-97.

Cavallo, J.D., Chardon, H., Chidiac, C., Courvalin, P., Dabernat, H., Drugeon, H., Dubreuil, L., Goldstein, F., Guery, B., Jarlier, V., Lambert, T., 2007. Recommandations. 2007. Comité de

l'Antibiogramme de la Société Française de Microbiologie, Paris, France.

Cederbrant, G., Kahlmeter, G., Ljungh, Â., 1993.The E test for antimicrobial susceptibility testing of *Helicobacter pylori*. J. Antimicrob. Chemother. 31(1):65-71.

DeCross, A.J., Marshall, B.J., McCallum, R.W., Hoffman, S.R., Barrett, L.J., Guerrant, R.L., 1993. Metronidazole susceptibility testing for *Helicobacter pylori:* comparison of disk, broth, and agar dilution methods and their clinical relevance. J. Clin. Microbiol. 31(8):1971-4.

Dharmalingam, S., Rao, U.A., Jayaraman, G., Thyagarajan, S.P., 2003. Relationship of plasmid profile with the antibiotic sensitivity pattern of *Helicobacter pylori* isolates from peptic ulcer disease patients in Chennai. Indian J Med Microbiol. 21(4):257-61

Dinić, M., Tasić, G., Stanković-Đorđević, D., Otašević, Lj., Tasić, M., Karanikolić, A. 2007. Serum anti-*Helicobacter pylori* IgA and IgG antibodies in asymptomatic children in Serbia. Scand J Infect Dis, 39:303-7

Dixon, M.F., Genta, R.M., Yardley, J.H., Correa, P., 1996. Classification and grading of gastritis: the updated Sydney system. The American journal of surgical pathology. 20(10):1161-81.

Dorji, D., Dendup, T., Malaty, H.M., Wangchuk, K., Yangzom, D., Richter, J.M., 2014. Epidemiology of *H. pylori* in Bhutan: the role of environment and Geographic location. Helicobacter. 19(1):69-73.

Drumm, B., Koletzko, S., Oderda, G., 2003. *Helicobacter pylori* infection in children: a consensus statement. Medical Position Paper: A report of the European Pediatric Task Force on Helicobacter pylori on a consensus conference. Budapest, Hungary, September 1998. J Pediatr Gastroenterol. 30: 207-213.

Fayed, S. S., Abd, E.L Dayem, Hussein, H., Sherief, M., S El, Naghi. 2011. Detection of virulent strains of *Helicobacter pylori* and its relation to symptoms and signs children. Asian Pac J Trop Med. 3(1):59-62.

Frenck, R.W., Clemens, J., 2003. *Helicobacter* in the developing world. Microbes Infect 5: 705–713.

Graham, D.Y., 1991. *Helicobacter pylori*: its epidemiology and its role in duodenal ulcer disease. J Gastroenterol Hepatol. 6: 105–113.

Graham, D.Y., Adam, E., Reddy, G.T., Agarwal, J.P., Agarwal, R., Evans, D.J., Malaty, H.M., Evans, D.G., 1991. Seroepidemiology of *Helicobacter pylori* infection in India. Dig Dis Sci. 36(8):1084-8.

Hashemi, M.R., Rahnavardi, M., Bikdeli, B., Zahedani, M.D., 2006. *H. pylori* infection among 1000 southern Iranian dyspeptic patients. World J Gastroenterol. 12(34): 5479–5482

Hussein, N.R., 2010. *Helicobacter pylori* and gastric cancer in the Middle East: a new enigma. World J Gastroenterol. 16(26):3226-34.

Iman, N., Khan, H., Iqbal, S., Rehman, S., 2008. Frequency of *H. pylori* in patients with upper GI symptoms. J Med Sci. 2008; 16(1):1-3.

Jemilohun, A.C., Otegbayo, J.A., Ola, S.O., Oluwasola, O.A., Akere, A., 2010. Prevalence of *H. pylori* among Nigerian patients with dyspepsia in Ibadan. Pan Afr Med J. 6(8):1-8.

Jorgensen, J.H., Turnidge, J.D., 2015. Susceptibility test methods: dilution and disk diffusion methods. In Manual of Clinical Microbiology, Eleventh Edition. American Society of Microbiology. pp. 1253-1273.

Khalifa, M.M., Sharaf, R.R., Aziz, R.K., 2010. *Helicobacter pylori*: a poor man's gut pathogen. Gut pathogens. 2(2):1-12

Kivi, M., Johansson, A.L., Reilly, M., Tindberg, Y., 2005. *Helicobacter pylori* status in family members as risk factors for infection in children. Epidemiol Infect. 133(04):645-52.

Koneman, E.W., 1997. Color atlas and textbook of diagnostic microbiology. Lippincott-Raven Publishers; 1997. p.539-76.

Lee, S.Y., 2014. Current progress toward eradicating *Helicobacter pylori* in East Asian countries: differences in the 2013 revised guidelines between China, Japan, and South Korea. World J Gastroenterol 20: 1493-1502.

Maaroos, H.I., Kekki, M., Villako, K., Sipponen, P., Tamm, A., Sadeniemi, L., 1990. The occurrence and extent of *Helicobacter pylori* colonization and antral and body gastritis profiles in an Estonian population sample. Scand. J. Gastroenterol. 25(10):1010-7.

Malekzadeh, R., Sotoudeh, M., Derakhshan, M.H., Mikaeli, J., Yazdanbod, A., Merat, S., Yoonessi, A., Tavangar, M., Abedi, B.A., Sotoudehmanesh, R., Pourshams, A., 2004. Prevalence of gastric precancerous lesions in Ardabil, a high incidence province for gastric adenocarcinoma in the northwest of Iran. J. Clin. Pathol. 57(1):37-42.

Massarrat, S., Saberi-Firoozi, M., Soleimani, A., Himmelmann, G.W., Hitzges, M., Keshavarz, H., 1995. Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. Eur. J. Gastroenterol. Hepatol. 7(5):427-33.

Matuschek, E., Brown, D.F., Kahlmeter, G., 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clinical Microbiology and Infection. 20(4): 0255-66.

Opekun, A.R., Gilger, M.A., Denyes, S.M., Nirken, M.H., Philip, S.P., Osato, M.S., Malaty, H.M., Hicks, J., Graham, D.Y., 2000. *Helicobacter pylori* infection in children of Texas. Journal of pediatric gastroenterology and nutrition. 31(4):405-10.

Osato, M.S., 2000. Antimicrobial susceptibility testing for *Helicobacter pylori*: sensitivity test results and their clinical relevance. Curr. Pharm. Des. 6(15):1545-55.

Ozbey, G., Bahcecioglu, I.H., Acik, M.N., 2012. Resistance rates to various antimicrobial agents of *Helicobacter pylori* isolates in Eastern Turkey. Inter J Mol Clin Microbiol. 2:148-52.

Pandeya, N., Whiteman, D.C., 2011. Prevalence and determinants of *Helicobacter pylori* sero-positivity in the Australian adult community. J Gastroenterol Hepatol. 26(8):1283-9

Perez-Perez, G.I., Rothenbacher, D., Brenner, H., 2004. Epidemiology of *Helicobacter pylori* infection. Helicobacter. 9(1):1-6.

Petrović, M., Artiko, V., Novosel, S., Ille, T., Šobić-Šaranović, D., Pavlović, S., Jakšić, E., Stojković, M., Antić, A., Obradović, V., 2010. Relationship between *H. pylori* infection estimated by 14C-urea breath test and gender, blood groups and Rhesus factor. Hellenic journal of nuclear medicine. 14(1):21-4.

Sasidharan, S., Uyub, A.M., Azlan, A.A., 2008. Further evidence of ethnic and gender differences for *Helicobacter pylori* infection among endoscoped patients. Trans. R. Soc. Trop. Med. Hyg 102(12):1226-32.

Sharma, S., Prasad, K.N., Chamoli, D., Ayyagari, A., 1995. Antimicrobial susceptibility pattern & biotyping of *Helicobacter pylori* isolates from patients with peptic ulcer diseases. Indian J Med Res. 102:261-6.

Shuker, W.S.H., 2007. Immune response in patients infected with *Helicobacter pylori*, Entamoeba histolytica and Giardia lamblia. Ph.D. Thesis. College of Medicine University of Baghdad.

Singh, V., Trikha, B., Nain, C.K., Singh, K., Vaiphei, K., 2002. Epidemiology of *Helicobacter pylori* and peptic ulcer in India. J Gastroenterol Hepatol. 17(6):659-65.

Smith, J.G., Li, W., Rosson, R.S., 2009. Prevalence, clinical and endoscopic predictors of *H. pylori* infection in an urban population. Connecticut medicine. 73(3):133-7.

Soltesz, V.A., Zeeberg, B.A., Wadström, T., 1992. Optimal survival of *Helicobacter pylori* under various transport conditions. Journal of Clinical Microbiology. 30(6):1453-6.

Suerbaum, S., Michetti, P., 2002. *Helicobacter pylori* infection. N Engl J Med. 347(15): 1175–1186.

Talley, N.J., Silverstein, M.D., Agreus, L., Nyren, O., Sonnenberg, A., Holtmann, G., 1998.AGA technical review: evaluation of dyspepsia. Gastroenterology. 114(3):582-95.

Tayal, R., De, A., Sundaravadanan, S., Kumar, M., Baveja, S., 2015.Comparison of two transport media and three culture media for the primary isolation of *Helicobacter* pylori from perforated peptic ulcer biopsy specimens. Studies; 5(4):1-5.6:7.

Thillainayagam, A.V., Arvind, A.S., Cook, R.S., Harrison, I.G., Tabaqchali, S., Farthing, M.J., 1991. Diagnostic efficiency of an ultra-rapid endoscopy room tests for *Helicobacter pylori*. Gut. 32(5):467-9.

Tolia, V., Brown, El-Baba, M., Lin, CH., 2000. *Helicobacter pylori* culture and antimicrobial susceptibility from pediatric patients in Michigan. The Pediatric infectious disease journal. 19(12):1167-71.

Toledo, H., López-Solís, R., 2009. Tetracycline resistance in Chilean clinical isolates of *Helicobacter pylori*. Journal of antimicrobial chemotherapy. 65(3):470-3.

Vale, F.F., Vítor, J.M., 2010.Transmission pathway of *Helicobacter pylori*: does food play a role in rural and urban areas. INT J Food Microbiol. 138(1-2):1-12.

Vanderplas, Y., 2001. The role of *Helicobacter pylori* in paediatrics. Current Opinion Infect Dis. 14: 315–321.

Wang, G.E., Wilson, T.J., Jiang, Q., Taylor, D.E., 2001. Spontaneous mutations that confer antibiotic resistance in *Helicobacter pylori*. Antimicrobial agents and chemotherapy. 45(3):727-33.

Weyermann, M., Rothenbacher, D., Brenner, H., 2009. Acquisition of *Helicobacter pylori* infection in early childhood: independent contributions of infected mothers, fathers, and siblings. Am. J. Gastroenterol. 104(1):182-9.

World Gastroenterology Organization Global Guidelines.2010. *Helicobacter pylori* in developing countries WGO Global Guideline: 9 (3), 16-22.

Yakoob, J., Jafri, W., Abid, S., Jafri, N., Abbas, Z., Hamid, S., Islam, M., Anis, K., Shah, H.A., Shaikh, H., 2005. Role of rapid urease test and histopathology in the diagnosis of *Helicobacter pylori* infection in a developing country. BMC gastroenterology. 5(1): 5-38.

Yang, Y.J., Sheu, B.S., Lee, S.C., Yang, H.B., Wu, J.J., 2005. Children of Helicobacter pylori-infected dyspeptic mothers are predisposed to *H. pylori* acquisition with subsequent iron deficiency and growth retardation. Helicobacter. 10(3):249-55.