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ORIGINAL ARTICLE

The high sensitivity and specificity of rapid urease test in diagnosis of *Helicobacter pylori* infection in Moroccan children

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ABSTRACT

Background and Objectives: Infesting nearly 50% of the world's population, *Helicobacter pylori* are thought to cause peptic ulcers, as well as gastric adenocarcinoma. Several diagnostic methods are available to detect this bacterium; however, at least two must be used together for an accurate diagnosis. This study evaluated the use of rapid urease test for diagnosis of *H. pylori* infection in a pediatric population.
Materials and Methods: Five gastric biopsies were taken from children during a 2-year period for the purpose of histological, molecular, bacteriological culture, and rapid urease testing.
Results: Among 83 children, 38 were male, and 45 were female with an age ranging of 2 to 15 years. The infected group represented 31%. The rapid urease test had a sensitivity of 88.5%, a negative predictive value of 94%, a specificity of 84.2%, and a positive predictive value of 72%.
Conclusion: A rapid urease test may be appropriate for ruling out *H. pylori* infection after a negative result. The positive results however, may be confirmed by a second invasive test.

Keywords: *Helicobacter pylori*; Children; Invasive tests; Rapid urease test; Polymerase chain reaction; Histology; Biopsy culture

INTRODUCTION

Helicobacter pylori (H. pylori) are one of the most prevalent human pathogens, and it is also a key pathogenic bacterium in pediatric gastroenterology. It affects more than half of the world's population (1). This bacterium continues to be a major public health issue, and a major cause of chronic gastritis, peptic

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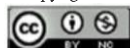
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ulcer disease, gastric adenocarcinoma. It is usually contracted during childhood, especially in developing countries, and persists throughout life if not treated (1). A study published in Pediatrics found that prevalence rates of *H. pylori* infection ranged from 1.8% to 65% among children (2). In developing countries, the prevalence is usually higher (up to 90% in some area) (3). In morocco, developing country, there is, however, limited information on the prevalence of *Helicobacter pylori* infection in children based on a review of published studies. In adults, Hasna et al. (4) showed a high prevalence of *H. pylori* infection in asymptomatic and gastric diseases patients and Bouihat et al. (5) reports a value of 69.4% detected by biopsies culture.

The identification of *H. pylori* in children has been widely discussed and differs from the diagnosis in adults (6). The clinical diagnosis of this infection in this age is difficult and the clinical manifestations have been often modest, and nonspecific (7). In clinical practice, the diagnostic tests are separated into two divisions: invasive tests and non-invasive tests. The non-invasive methods are based on the presence of bacterial enzymes, antigens, antibodies, or DNA sequences and include 13C or 14C urea breath test, stool antigen test (SAT), serology, and molecular methods (8). The invasive methods include endoscopy and gastric biopsy followed by histology or culture, or molecular methods based on biopsy samples. Each invasive test has a distinct clinical advantage. The rapid urease test (RUT) provides an opportunity to begin treatment immediately after the test. Histological examinations provide an assessment of gastric mucosa. A culture is especially useful when it comes to testing antibiotic susceptibility in advance of selecting an appropriate antibiotic eradication therapy. PCR is an emerging method for detecting the bacterium without culture (9).

Consequently, it is, therefore, an active debate as to what method should be used to diagnose *H. pylori* infection. The identification of this infection has examined several criteria, including sensitivity and specificity (10), and no single method can fulfill all of the criteria for acceptable sensitivity and specificity (11). The invasive methods (histology, culture, PCR, and RUT), based on the esophagogastroduodenoscopy were recommended as a first choice for the initial diagnosis (12). The non-invasive tests (stool antigen test, urea breath test, and serology) are, however, mainly used in epidemiological studies or for eradication control (6, 11).

Although, histology was the first method used for the detection of *H. pylori* providing additional and essential information on the status of the mucosa (13), the Rapid Urease Test (RUT) was currently proposed by several studies as an alternative method for diagnostic of *H. pylori.* It is also described to be very fast, inexpensive, reliable and simple technique (9, 14) providing a quick results in less than 24 hours (15). Furthermore, relying on histology as the only invasive test to determine *H. pylori* infection in pediatric clinical practice may not be sufficient. The reliability of this method is mainly determined by the number and location of specimens collected as well as the skills of the operator (16). Therefore, the primary objective of this study was to evaluate the performance of the in house RUT to detect *H. pylori* in biopsies from children.

MATERIALS AND METHODS

Patients and gastric biopsies collection. A cross-sectional study was carried out with a group of children from the Hassan II University Hospital in Fez, Morocco. During a two-year period, this study recruited randomly a children (under 16 years of age) who required diagnostic or therapeutic upper gastrointestinal endoscopy and had not taken a drug within 30 days of the testing (antibiotics or proton pump inhibitors). An informed consent form and a structured questionnaire containing sociodemographic and clinical information were obtained from the patient's parents. The study was approved by the Research Ethics Committee.

Histologic study. According to Zaitoun AM et al. (17), the biopsy specimens collected for histology (one antral and one corpus biopsy) were fixed in 10% formalin, embedded in paraffin, and cut at 6 µm. Sections were stained with hematoxylin–eosin–saffron for light microscopy. All the sections were observed by the pathologist who noted the presence or absence of *H. pylori.* The result was confirmed by another pathologist.

Culture, in house RUT and extraction of DNA. The culture was performed according to Mégraud F et al. (18) with modifications. Briefly, from the antrum, three gastric biopsies were taken and im-

mediately placed in a transport medium (Portagerm, BioMérieux) and kept frozen at 4 to 8°C. The Department of Microbiology processed the biopsy specimens within 24 hours of receiving them. A manual homogenizer (potter grounder) was used to homogenize all tissues in 1 ml of broth. Subsequently, this broth was vortexed vigorously and used immediately for culture, in house RUT and extraction of DNA.

For the culture, 0.1 ml from the suspension of biopsies was plated on Columbia agar with 5% sheep blood (Biomerieux, Marcy etoile, France), Pylori agar (Biomerion), and in 1 mL of urea broth. After incubation of the plates at 37°C in an aerobic micro-atmosphere (5% O_2, 10% CO_2, and 85% N_2) the colonies suspected to be *H. pylori* were identified by colony morphology (gray, small, and translucent colonies after 3 to 4 days of incubation), positive biochemical reactions (cytochrome oxidase, catalase, and urease), and Gram staining (curved rod) (18).

For the in house RUT, 0.1 ml from the suspension of biopsies was immersed in a tube containing 1ml of the urease test reagent. The test was read both within 2 h and after overnight incubation at 37°C, and was considered positive when changing the colour from yellow/orange to pink/purple in less than 24 h of incubation (Fig. 1) (19).

For the extraction of DNA, and following the manufacturer's instructions, the QIAamp DNA mini kit (Qiagen, Germany) was used to separate genomic DNA from the suspension of biopsies. This DNA was stored at -20°C for until use in molecular study.

Molecular study. The gene of ureC was used in this study to detect the *H. pylori* bacteria. The oligonucleotide primer pair is specific for a fragment of the urease C (20). PCR reaction was carried out in a final volume of 50 µL as described by Pandya et al. (21). The Table 1 summarizes the primer sequences, expected product size, and PCR conditions. The PCR product was run on a 1.5% agarose gel containing ethidium bromide and visualized under ultraviolet light (Fig. 2).

***H. Pylori* status (Gold standard definition).** To confirm a positive *H. pylori* status, the gold standard used in this study was based on a positive culture result or a both positive result of histology and PCR. A subject who did not fit these diagnostic criteria was considered as *H. pylori* negative.

Statistical analysis. For categorical data, the mean age and the proportions were calculated using a simple descriptive analysis. The sensitivity, specificity, positive and negative predictive values were calculated using a standard method and the $\chi2$ test.

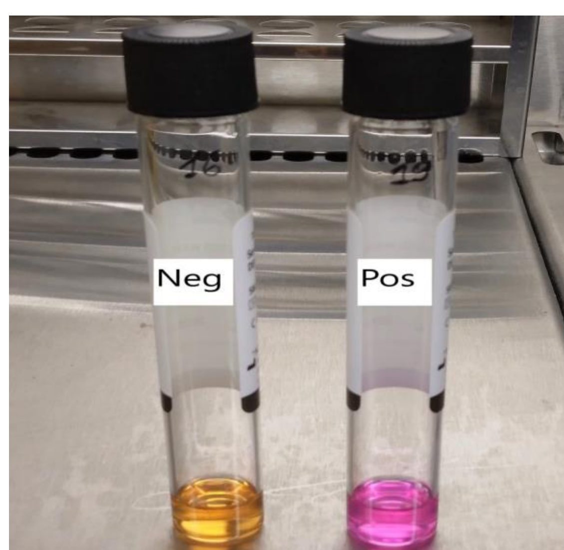

Fig. 1. a positive (Pos) and negative (Neg) result of in house RUT

Fig. 2. ureC gene bands on agarose gel 1.5%

Table 1. PCR conditions and the primer sequence of target gene used in study (ureC, 294 bp)

Primer sequence	PCR conditions	Reference
F-5′-AAGCTTTTAGGGGTGTTAGGGGTT-3′ R-3′-AAGCTTACTTTCTAACACTAACGC-5	denaturation for 1 minute at 93°C; annealing for 1 minute at 55°C and extension for 1 minute at 72°C (35 cycles), size of the PCR product	(22)

RESULTS

The patients' demographic and clinical characteristics are shown in the Table 2. A total of 83 children, 38 males (45.8%), and 45 females (54.23%), from the Fez –Meknes region of Morocco were included in the study. The number of the patients living in urban area was 52 (62.7%). The age of the subjects was ranged

Table 2. demographic and clinical characteristics of patients

		Number	Percentage
Age (years)	< 4	20	24.1%
	4 - 6	15	18.1%
	7 - 9	13	15.7%
	10 - 13	24	28.9%
	>14	11	13.3%
Father's education	No	33	39.8%
	Yes	50	60.2%
Mother's education	No	40	48.2%
	Yes	43	51.8%
Residence	Urban	52	62.7%
	Rural	31	37.3%
Access to sanitary water in a residence	No	14	16.9%
	Yes	69	83.1%
Socioeconomic level	Low	51	61.4%
	Medium	29	34.9%
	High	3	3.6%
Sharing the bad with parents	No	8	9.6%
	Yes	75	90.4%
Anaemia	No	76	91.6%
	Yes	7	8.4%
Delayed growth	No	78	94.0%
	Yes	5	6.0%
Suspicion of celiac disease	No	64	77.1%
	Yes	19	22.9%
Chronic diarrhoea	No	76	91.6%
	Yes	7	8.4%
Abdominal pain	No	76	91.6%
	Yes	7	8.4%
Dysphagia	No	75	90.4%
	Yes	8	9.6%
Gastro-oesophageal reflux	No	80	96.4%
	Yes	3	3.6%
Persistent vomiting	No	68	81.9%
	Yes	15	18.1%
Upper gastrointestinal bleeding	No	79	95.2%
	Yes	4	4.8%
Gastritis erythema	No	68	81.9%
	Yes	15	18.1%
Nodular gastritis	No	66	79.5%
	Yes	17	20.5%
ulcer gastritis	No	81	97.6%
	Yes	2	2.4%

from 2 to 15 years being a mean of 7.7 years.

In this study, biopsies of all children were tested using invasive methods. Based on the results of biopsies culture, histology, PCR, and in-house RUT (Table 3), the *H. pylori* was found in 15/83 (18%), 29/83 (35%), 29/83 (35%), and 32/83 (39%) of children. The lowest rate of *H. pylori* infection was found by biopsy culture followed by that found in the histology and the PCR test. The highest rate was found by the in-house RUT. However, according to the results of gold standard described above, the frequency of *H. Pylori* infection in our group was 31% (26 children out of 83).

Compared to the gold test results (Table 4), the in-house RUT has a high sensitivity (88.5%), a negative predictive value of 94%, a true negative value of 48, and a false negative value of 3. However, a good specificity (84.2%) and a moderate positive predictive value of 72%, 23 true positives and 9 false positives were detected in this test

DISCUSSION

In our context the use of histology as a single invasive test may be insufficient and the use of a rapid and economical another invasive test is needed for an appropriate diagnosis. However, choosing between invasive tests (culture, PCR, or RUT) can be difficult, since each one of them has its own advantage and disadvantage. The culture, which appears in several studies, should be an option, but due to the expense, the need for specialized transport, and the requirement of high-tech techniques, its sensitivity could be compromised (23). PCR tests, which are capable of identifying infections in children with acceptable sensitivity, are not widely available for clinical use. It is also extremely expensive and generally used only at tertiary levels (13). The RUT, however, is an optimal methods, it is commonly used to detect *H. pylori* infection on gastric biopsy specimens because the results can be interpreted easily and rapidly. Moreover, it is pertinent to note that many kits of RUT were available with highly sensitives and specific results, but the cost is, however, relatively expensive (24). It is therefore necessary to have a rapid and economical RUT available to make an accurate diagnosis. The purpose of the study was to propose and evaluate an in-house RUT for quick and economical diagnosis of *H. pylori* infection based on the gold standard.

The authors agreed that confirmation of infection based on a single test increases the error rate. It should depend on results of the combinations of different tests, as often used in several clinical researches, such as association of urease and histology results (19) or of histology and Immunohistochemistry (IHC) results (25). But this artifice can fail, leading to an error when determining whether the patient is infected or not. Thus, deciding which method will be the gold standard and how many will be needed is very critical.

In This study, and as mentioned by several authors (9, 11, 26-28), we used positive culture or both positive PCR and Histology as a gold standard. Positive cultures reduce false positives since they are consid-

Table 3. Results of detection of *H. pylori* by each test used in the study

	In house RUT n=83	**HE n=83**	**BC n=83**	**PCR n=83**	**Gold test n=83**
NEGATIVE	51 (61%)	54 (65%)	68 (82%)	54 (65%)	57 (69%)
POSITIVE	32 (39%)	29 (35%)	15 (18%)	29 (35%)	26 (31%)

HE (hematoxylin and eosin); BC (biopsies culture)

Table 4. Results of the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of in house RUT according to the 'gold standard' results

		gold test		**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Sig**
		Negative	**Positive**	**(%)**	**(%)**	**(%)**	**(%)**	
In house RUT	Negative	48	3	88.5	84.2	72	94	<0.0001
	Positive	9	23					

ered the most reliable indicator of *H. Pylori* infection and only this method is 100% specific. Furthermore, when the culture was negative, a positive PCR and histology test may increase the true positive and decrease false-negative cases. This association improve the specificity of our gold test.

However, despite its high specificity, our gold test would invariably produce a number of false-negative results; since the sensitivity of culture depends on the expertise of the laboratory and the quality of the culture media. As well as the sensitivity of histology, which is affected by many factors such as the staining technique, and the experience of the pathologist (8, 13). Moreover, our laboratory used a simple stain method (hematoxylin and eosin) for its histological examinations, which may increase the number of false negatives in the gold standard. Nevertheless, two different staining methods should be used: hematoxylin and eosin for the assessment of inflammatory cells, and Giemsa for the determination of pathogens (17).

According to the results of gold test, the *H. pylori* infection identified in 31% of our study group was higher comparing with that reported by authors in other context. In pediatric and adolescent Brazilian population (29), in a group of Saudi Arabian school students (30), in Iran (31), in Sudan (32) and in Jordan (33) the frequency of *H. pylori* infection was respectively, 24.7%, 27.4%, 19.8%, 8% and 14.6%. In Moroccan adult group, *H. pylori* was detected in 87 (57.24%), and 69 (45.10%) cases by histology, and PCR, respectively (34), in 75.5% among 324 patients (35) and in 69.9% of 429 patients (36). The value of this variable (prevalence of *H. pylori* infection) differed from country to country due to the level of urbanization, sanitation, access to clean water, socioeconomic status and a range of behavioural risk factors (12, 26). Out of risk factors mentioned by authors, the lower socio-economic status and the sharing the beds with parents or siblings were mostly identified in our study group and may be increase the frequency of *H. pylori* infection in our population.

Consistent with previous studies the in house RUT found the high sensitivity in our population (88.5%). In Taiwan (37), and in Barcelona, Spain (38) the sensitivity of in house RUT was respectively 91%, and 91%. There was also, in this study, the lowest number of false negative, only 3 cases, with a higher PVN of 94%. Several factors may be decrease a number of false negative in our population, such the exclusion of children who confirmed that they took drugs during the 30 days preceding the endoscopy; as well as the in-house RUT was done on the basis of three biopsies. According to research, increasing the number of biopsies and the density of bacteria increased RUT sensitivity (28). In a study included 116 patients, the sensitivity increased from 61% using one biopsy to 74% using four antral biopsies (39). Researchers found similar results when comparing the sensitivity of RUT when using one piece of biopsy in comparison to more than one piece of biopsy from the gastric antrum in children by Seo et al. (40).

This study found that the in-house RUT, compared with the gold standard, had moderate specificity (84.2%). This value was slightly lower than that mentioned by authors who generally provided a value of over 90% (28). However, our value was higher than that of other studies such 65% reported by Hasosah et al. in Saudi Arabian children using similar gold test (27). The in-house RUT shows a significant number of false-positive results of 11 cases (26.2%) with a low VPP (73.8%). According to Osaki et al. (41), this result could be explained by the presence of other urease-containing organisms such as *Proteus mirabilis, Citrobacter freundii, Klebsiella pneumonia, Enterobacter cloacae* and the *Staphylococcus aureus.* Furthermore, the reading delay can reduce the specificity of our test. It has been confirmed by several authors that RUT samples should not be used to determine treatment after 24 hours to avoid false positive detections (42).

In summary, this study demonstrated that the in house RUT was confidant for detecting negative cases. However, this confidence goes down were the positive result was given. In another hand, the diagnostic of a positives result should be associated with the endoscopic diagnosis of gastric status (43). The author confirmed that nodular gastritis may be a pathognomonic endoscopic finding of childhood *H. pylori* infection (44%-67% in children vs 0.19%-13% in adults) (43). This study showed that the nodular gastritis and erythema gastritis was the most frequent endoscopic diagnostic founded in the children. Similar result was reported by Kalach N et al. (43).

CONCLUSION

It is relevant to note that several studies have been performed to evaluate detection tests for *H. pylori* in-

fection. To the best of our knowledge, this is the first study of its kind in our context. We found that the in-house RUT has a higher sensitivity and moderate specificity, making it an appropriate test for denying *H. pylori* infection in our population. Its value as a screening test was confirmed especially when giving a negative result. Nevertheless, the positive result should be confirmed by a second test, such as histology. The protocol used in this study has the advantage of allowing in-house PCR, culture and molecular analysis to be performed on the same tissue, thus making exploring the resistance to antibiotics possible (42, 44).

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REFERENCES

1. Kotilea K, Kalach N, Homan M, Bontems P. *Helicobacter pylori* infection in pediatric patients: update on diagnosis and eradication strategies. *Paediatr Drugs* 2018; 20: 337-351.
2. Roma E, Miele E. *Helicobacter pylori* infection in pediatrics. *Helicobacter* 2015; 20 Suppl 1: 47-53.
3. Hestvik E, Tylleskar T, Kaddu-Mulindwa DH, Ndeezi G, Grahnquist L, Olafsdottir E, et al. *Helicobacter pylori* in apparently healthy children aged 0-12 years in urban Kampala, Uganda: a community-based cross sectional survey. *BMC Gastroenterol* 2010; 10: 62.
4. Hasna B, Imane E, Ghizlane B, Reda JM, Abdeljabbar M, Hassan J, et al. Epidemiological study of *Helicobacter pylori* infection and its association with gastric carcinogenesis. *Gulf J Oncolog* 2021; 1: 7-13.
5. Bouihat N, Burucoa C, Benkirane A, Seddik H, Sentissi S, Al Bouzidi A, et al. *Helicobacter pylori* primary antibiotic resistance in 2015 in Morocco: A phenotypic and genotypic prospective and multicenter study. *Microb Drug Resist* 2017; 23: 727-732.
6. Sabbagh P, Javanian M, Koppolu V, Vasigala VR, Ebrahimpour S. *Helicobacter pylori* infection in children: an overview of diagnostic methods. *Eur J Clin Microbiol Infect Dis* 2019; 38: 1035-1045.
7. Burucoa C. Diagnostic tests for *Helicobacter pylori* infection in children. *Arch Pediatr* 2010; 17: 814-815.
8. Braden B. Diagnosis of *Helicobacter pylori* infection. *BMJ* 2012; 344: e828.
9. Sabbagh P, Mohammadnia-Afrouzi M, Javanian M, Babazadeh A, Koppolu V, Vasigala VR, et al. Diagnostic methods for *Helicobacter pylori* infection: ideals, options, and limitations. *Eur J Clin Microbiol Infect Dis* 2019; 38: 55-66.
10. Kraft C, Stack A, Josenhans C, Niehus E, Dietrich G, Correa P, et al. Genomic changes during chronic *Helicobacter pylori* infection. *J Bacteriol* 2006; 188: 249-254.
11. Kotilea K, Bontems P, Touati E. Epidemiology, diagnosis and risk factors of *Helicobacter pylori* infection. *Adv Exp Med Biol* 2019; 1149: 17-33.
12. 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: 991-1003.
13. Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of *Helicobacter pylori*: What should be the gold standard? *World J Gastroenterol* 2014; 20: 12847-12859.
14. Monteiro L, Oleastro M, Lehours P, Mégraud F. Diagnosis of *Helicobacter pylori* infection. *Helicobacter* 2009; 14 Suppl 1: 8-14.
15. Chey WD, Wong BCY, Gastroenterology PPC of the AC of. American college of gastroenterology guideline on the management of *Helicobacter pylori* infection. *Am J Gastroenterol* 2007; 102: 1808-1825.
16. Hirschl AM, Makristathis A. Methods to detect *Helicobacter pylori*: From culture to molecular biology. *Helicobacter* 2007; 12 Suppl 2: 6-11.
17. Akeel M, Elhafey A, Shehata A, Elmakki E, Aboshouk T, Ageely H, et al. Efficacy of immunohistochemical staining in detecting *Helicobacter pylori* in Saudi patients with minimal and atypical infection. *Eur J Histochem* 2021; 65: 3222.
18. Mégraud F, Lehours P. *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 2007; 20: 280-322.
19. Ramis IB, De Moraes EP, Fernandes MS, Mendoza-Sassi R, Rodrigues O, Juliano CRV, et al. Evaluation of diagnostic methods for the detection of *Helicobacter pylori* in gastric biopsy specimens of dyspeptic patients. *Braz J Microbiol* 2012; 43: 903-908.
20. Diouf A, Martinez-Gomis J, Miquel M, Quesada M, Lario S, Sixou M. Comparaison de quatre paires d'amorces différentes dans la détection d'*Helicobacter pylori* dans les biopsies gastriques et les prélèvements oraux. *Pathol Biol* 2009; 57: 30-35.
21. Pandya HB, Agravat HH, Patel JS. Prevalence of Specific *Helicobacter pylori* CagA, VacA, IceA, UreC Genotypes and its Clinical Relevance in the Patients with Acid-Peptic Diseases. *J Clin Diagn Res* 2017; 11:

DC23-DC26.
22. Kalaf EA, Al-Khafaji ZM, Yassen NY, Al-Abbudi FA, Sadwen SN. Study of the cytoxin-associated gene a (CagA gene) in *Helicobacter pylori* using gastric biopsies of Iraqi patients. *Saudi J Gastroenterol* 2013; 19: 69-74.
23. Chisholm SA, Owen RJ. Application of polymerase chain reaction-based assays for rapid identification and antibiotic resistance screening of *Helicobacter pylori* in gastric biopsies. *Diagn Microbiol Infect Dis* 2008; 61: 67-71.
24. Lim L-L, Ho K-Y, Ho B, Salto-Tellez M. Effect of biopsies on sensitivity and specificity of ultra-rapid urease test for detection of *Helicobacter pylori* infection: A prospective evaluation. *World J Gastroenterol* 2004; 10: 1907-1910.
25. Miftahussurur M, Yamaoka Y. Diagnostic methods of *Helicobacter pylori* infection for epidemiological studies: Critical importance of indirect test validation. *Biomed Res Int* 2016; 2016: 4819423.
26. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of *Helicobacter pylori* infection: Systematic review and meta-analysis. *Gastroenterology* 2017; 153: 420-429.
27. Hasosah M. Accuracy of invasive and noninvasive methods of *Helicobacter pylori* infection diagnosis in Saudi children. *Saudi J Gastroenterol* 2019; 25: 126-131.
28. Thaker Y, Moon A, Afzali A. *Helicobacter pylori*: A review of epidemiology, treatment, and management. *J Clin Gastroenterol Treat* 2016; 2: 019.
29. Toscano EP, Madeira FF, Dutra-Rulli MP, Gonçalves LOM, Proença MA, Borghi VS, et al. Epidemiological and clinical-pathological aspects of *Helicobacter pylori* infection in Brazilian children and adults. *Gastroenterol Res Pract* 2018; 2018: 8454125.
30. Telmesani AMA. Helicobacter pylori: Prevalence and relationship with Abdominal pain in School Children in Makkah City, Western Saudi Arabia. *Saudi J Gastroenterol* 2009; 15: 100-103.
31. 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.
32. Abbas M, Sharif FA, Osman SM, Osman AM, El Sanousi SM, Magzoub M, et al. Prevalence and associated symptoms of *Helicobacter pylori* infection among Schoolchildren in Kassala State, East of Sudan. *Interdiscip Perspect Infect Dis* 2018; 2018: 4325752.
33. Altamimi E, Alsharkhat N, Aljawarneh A, Abu Hamad MDR, Assi AA, Alawneh S, et al. Declining prevalence of *Helicobacter pylori* infection in Jordanian children, report from developing country. *Heliyon* 2020; 6(7): e04416.
34. El Khadir M, Alaoui Boukhris S, Benajah D-A, El Rhazi K, Ibrahimi SA, El Abkari M, et al. Detection of *Helicobacter pylori* urease antigen in saliva in patients with different gastric *H. pylori* status. *J Chin Med Assoc* 2016; 79: 363-367.
35. Benajah DA, Lahbabi M, Alaoui S, El Rhazi K, El Abkari M, Nejjari C, et al. Prevalence of *Helicobacter pylori* and its recurrence after successful eradication in a developing nation (Morocco). *Clin Res Hepatol Gastroenterol* 2013; 37: 519-526.
36. Alaoui Boukhris S, Benajah D-a, El Rhazi K, Ibrahimi SA, Nejjari C, Amarti A, et al. Prevalence and distribution of *Helicobacter pylori* cagA and vacA genotypes in the Moroccan population with gastric disease. *Eur J Clin Microbiol Infect Dis* 2012; 31: 1775-1781.
37. Fan C-C, Chen C-H, Chou C, Kao T-Y, Cheng AN, Lee AY-L, et al. A time-saving–modified Giemsa stain is a better diagnostic method of *Helicobacter pylori* infection compared with the rapid urease test. *J Clin Lab Anal* 2020; 34(4): e23110.
38. Calvet X, Sánchez-Delgado J, Montserrat A, Lario S, Ramírez-Lázaro MJ, Quesada M, et al. Accuracy of Diagnostic Tests for *Helicobacter pylori:* A Reappraisal. *Clin Infect Dis* 2009; 48: 1385-1391.
39. Lee T-H, Lin C-C, Chung C-S, Lin C-K, Liang C-C, Tsai K-C. Increasing biopsy number and sampling from gastric body improve the sensitivity of rapid urease test in patients with peptic ulcer bleeding. *Dig Dis Sci* 2015; 60: 454-457.
40. Seo J-H, Park JS, Yeom JS, Lim J-Y, Park C-H, Woo H-O, et al. Correlation between positive rate and number of biopsy samples on urease test in childhood *Helicobacter pylori* infection. *J Korean Med Sci* 2014; 29: 106-109.
41. Osaki T, Mabe K, Hanawa T, Kamiya S. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of *Helicobacter pylori* infection. *J Med Microbiol* 2008; 57: 814-819.
42. Uotani T, Graham DY. Diagnosis of *Helicobacter pylori* using the rapid urease test. *Ann Transl Med* 2015; 3: 9.
43. Kalach N, Bontems P, Raymond J. *Helicobacter pylori* infection in children. *Helicobacter* 2017; 22 Suppl 1: 10.1111/hel.12414.
44. Li Y, Rimbara E, Thirumurthi S, Trespalacios A, Reddy R, Sabounchi S, et al. Detection of clarithromycin resistance in *Helicobacter pylori* following noncryogenic storage of rapid urease tests for 30 days. *J Dig Dis* 2012; 13: 54-59.