Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.484

World J Gastroenterol 2009 January 28; 15(4): 484-488
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Application of Stool-PCR test for diagnosis of *Helicobacter pylori* infection in children

Tahereh Falsafi, Raha Favaedi, Fatemeh Mahjoub, Mehri Najafi

Tahereh Falsafi, Raha Favaedi, Department of Biology, Alzahra University, 1993891176 Tehran, Iran
Fatemeh Mahjoub, Mehri Najafi, Department of Gastroenterology and Pathology, Medical Center for Children, Tehran 15614, Iran
Author contributions: Falsafi T designed the study and wrote the manuscript, also provided vital analytical tools; Favaedi R performed the majority of experiments; Najafi M provided the biopsies and patient related information; Mahjoub F performed histopathological study of the biopsies.
Correspondence to: Tahereh Falsafi, Department of Biology, Azzahra University, Deh Vanak, 1993891176 Tehran, Iran. tfalsafi@yahoo.com
Telephone: +98-21-88058912 Fax: +98-21-88058912
Received: November 12, 2008 Revised: December 25, 2008
Accepted: December 31, 2008
Published online: January 28, 2009

Abstract

AIM: To evaluate the usefulness of stool-PCR test for diagnosis of *Helicobacter pylori* (*H pylori*) infection in pediatric populations.

METHODS: Based on endoscopic features (including nodular gastritis, erosive duodenitis and ulcer) and/or a positive rapid urease test (RUT) obtained during endoscopy, 28 children from a group of children admitted to the Children's Medical Center of Tehran for persistent upper gastrointestinal problems were selected to compare biopsy-based tests with stool-PCR. Their gastric activity and bacterial density were graded by the updated Sydney system, and their first stool after endoscopy was stored at -70℃. Biopsies were cultured on modified campy-blood agar plates and identified by gram-staining, biochemical tests, and PCR. Two methods of phenol-chloroform and boiling were used for DNA extraction from *H pylori* isolates. Isolation of DNA from stool was performed using a stool DNA extraction kit (Bioneer Inc, Korea). PCR was performed using primers for detection of *vacA*, *cagA*, and *16srRNA* genes in both isolates and stool.

RESULTS: Sixteen out of 28 child patients (57%) were classified as *H pylori* positive by biopsy-based tests, of which 11 (39%) were also positive by stool-PCR. Sensitivity and specificity of stool-PCR was 62.5% and 92.3% respectively. *H pylori* was observed in histological sections for 10 out of 11 stool-positive patients. Association was observed between higher score of *H pylori* in histology and positivity of stool-PCR. Also association was observed between the more severe form of gastritis and a positive stool-PCR.

CONCLUSION: Association between higher score of *H pylori* in histology and a positive stool-PCR make it a very useful test for detection of *H pylori* active infection in children. We also suggest that a simple stool-PCR method can be a useful test for detection of *H pylori* virulence genes in stool.

Key words: *Helicobacter pylori*; Non-invasive diagnosis; Stool-PCR; Histology; Score; Children; Iran

Peer reviewer: Vasiliy I Reshetnyak, MD, PhD, Professor, Scientist Secretary of the Scientific Research Institute of General Reanimatology, 25-2, Petrovka str., 107031, Moscow, Russia

Falsafi T, Favaedi R, Mahjoub F, Najafi M. Application of Stool-PCR test for diagnosis of *Helicobacter pylori* infection in children. *World J Gastroenterol* 2009; 15(4): 484-488 Available from: URL: http://www.wjgnet.com/1007-9327/15/484.asp DOI: http://dx.doi.org/10.3748/wjg.15.484

INTRODUCTION

Helicobacter pylori (*H pylori*) infection in humans is associated with gastritis, gastric ulcer, and gastric cancers[1,2]. Infection occurs mainly in childhood and infected individuals usually carry it for life unless treated[3,4]. Epidemiology of infection by *H pylori* has been characterized by a linear increase with age in western industrial countries and by a large number of children and juveniles being infected in developing countries[5]. Currently used methods for diagnosis of *H pylori* infection, such as culture, histology, and rapid urease test (RUT) are very sensitive and highly specific tests, but require invasive sampling. The non-invasive methods, such as serology and urea breath test (UBT), are also sensitive and specific; however, positive results obtained by serology do not necessarily indicate current infection by *H pylori*[6,7]. UBT requires an expensive instrument, which is not always available in routine clinical laboratories, especially in developing countries. In addition the performance of the test has been associated with

some disadvantages for infants and very young children, as well as patients with certain neurological disorders[6,7]. *H pylori* is not an intestinal pathogen, and therefore is expected to be present in low concentrations in stool; however, it can be detected in stool specimens by *H pylori* stool-antigen (HpSA) test, PCR, or even culture[8-12]. The HpSA test has been shown to be very useful, especially in children; however, various commercial tests have shown some discrepancies in different geographical areas[13-15]. Stool-culture is a very specific method; however, the massive numbers of diverse micro-organisms in stool makes it very difficult in routine practice[8,12]. Stool-PCR may also be a very useful method in detection of *H pylori* infection, but reported success rates for the detection of *H pylori* DNA in feces vary from 25% to 100%[6,8]. This variability is probably due to *H pylori* degradation in the gastrointestinal tract and/or the presence of inhibitors such as complex polysaccharides[16,17]. The purpose of this study was to evaluate the usefulness of the stool-PCR test for diagnosis of *H pylori* infection in pediatric populations.

MATERIALS AND METHODS

Patients

Based on endoscopic features (including nodular gastritis, erosive duodenitis, or ulcers) and/or a positive rapid urease test obtained during endoscopy, 28 children from a group of children admitted to a children's medical center in Tehran for persistent upper gastrointestinal problems were selected to compare biopsy-based tests and stool-PCR. Of these patients, two antral biopsies similar to that of RUT were obtained for culture and histology, and the first stool after endoscopy but before antibiotic therapy was collected and stored at -70℃. These children were asked to have a vegetable free diet 24 h before sampling. Stool samples were also collected from a few healthy children that showed no symptoms. Patients who tested positive by culture or positive by both RUT and histology were considered as positive controls and those who tested negative by all three endoscopy-based tests were considered as negative ones.

Biopsy-based tests

Culture of biopsy samples was performed as previously described[12,18]. Briefly, antral biopsies were placed in a modified campy-thio medium and incubated at 37℃ under a micro-aerobic atmosphere. After 3 d, 20 μL of the enrichment culture was streaked onto modified campy-blood agar and incubated for 5-10 d until colonies were evident. The grown colonies were identified by gram-staining, oxidase, urease, and nitrate-reduction tests.

RUT was performed using urea broth as previously described. The RUT result was read either within 2 h at endoscopy room or after overnight incubation under a micro-aerobic atmosphere at 37℃ according to the previously described protocol[12,18]. Histological examination of the biopsies was performed after H&E, and Geimsa staining *H pylori* density, gastritis, and inflammation were graded according to the modified Sydney system[19,20]. The cases of gastritis with follicular formation were classified as follicular gastritis either with or without activity[20].

DNA extraction and PCR

Two methods of phenol-chloroform and boiling were used for DNA extraction from *H pylori* isolates. For the first one, a pool of colonies in 2 mL sterile 0.9% NaCL, was centrifuged at 10000 g, the pellet was resuspended in 400 μL of extraction buffer (10 mmol/L Tris-HCL, pH 8.0; 5 mmol/L EDTA, 0.1% sodium dodecyl sulfate), and proteinase K at final concentration of 0.5 mg/mL was added to homogenizates. Samples were incubated at 55℃ for 2-4 h before incubation at 95℃ for 10 min. DNA was purified by phenol-chloroform, precipitated by absolute ethanol at -20℃ in presence of 0.3 mol/L sodium acetate, pelleted by centrifugation at 12000 g for 30 min and allowed to dry in air. The pellet in sterile double-distilled water was quantified by measuring the optical density at 260 nm and stored at -20℃ until they were used as PCR templates. For the second method, a loopful of colonies was suspended in 1 mL of phosphate buffer saline (PBS, pH 7.6), washed by centrifugation at 14000 g for 2 min, and resuspended in 50 μL of sterile, double distilled water. Tubes were then boiled at 95℃ for five minutes and 2 μL of 1/5 dilution of this extract (containing approximately 20 ng of DNA) was immediately used as template for PCR. Isolation of DNA from stool was performed using a stool DNA extraction kit (Bioneer Inc, Korea), where substances inhibiting PCR were removed by filtration according to the manufacturer's instructions. Stool-PCR controls were 3 uninfected feces from the *H pylori*-negative patient (as determined by endoscopy-based tests) seeded or not seeded with known concentrations (equivalent to McFarland No. 5) of 26695 *H pylori* ATCC strain.

PCR primers (Faza Biotech Inc, Iran) were designed on the basis of published sequences of *H pylori 16SrRNA, vacA*, and *cagA*[8,21]. Table 1 resumes the sequences and experimental details for PCR.

RESULTS

The H pylori status

Sixteen out of 28 child patients (57%) were classified as *H pylori* positive by biopsy-based tests. Of 16 *H pylori* positive children 6 were positive by culture, 5 were positive by all of the 3 tests, and 5 were positive by RUT plus histology.

PCR results

DNA isolated from culture positive controls showed amplification for *H pylori* specific primer(s) including *vacA* (*s*, m), *cagA*, and *16srRNA*. Stool-PCR positive controls, which were 3 uninfected feces from the *H pylori*-negative patient containing known concentrations of 26695 *H pylori* ATCC strain, showed amplification for *H pylori* DNA only after purification by column chromatography procedure. No amplification was observed for the negative stool-PCR controls (stool

Table 1 Primers sequences and PCR conditions

Primers	Sequences	Product size (bp)	PCR conditions
16sRNA	5'GCTAAGAGATCAGCCTATGTCC3'	500	95℃ 5 min (1 cycle); 94℃ for 1 min, 55℃ for 1 min
	5'TGGCAATCAGCGTCAGGTAATG3'		72℃ for 2 min (39 cycles); 72℃ for 7 min
VacA (s)	5'ATGGAAATACAACAAACACAC3'	s1: 259	95℃ 4 min (1 cycle); 95℃ for 1 min, 52℃ for 1 min
	5'CTGCTTGAATGCGCCAAAC3'	s2: 286	72℃ for 1 min (35 cycles); 72℃ for 10 min
vacA (m)	5'CAATCTGTCCAATCAAGCGAG34	m1: 570	95℃ 4 min (1 cycle); 95℃ for 1 min, 52℃ for 1 min
	5'GCGTCTAAATAATTCCAAGG3'	m2: 642	72℃ for 1 min (35 cycles); 72℃ for 10 min
cagA	5'AATACACCAACGCCTCCA3'	400	94℃ for 4 min (1 cycle); 94℃ for 1 min, 59℃ for 1 min
	5'TTGTTGCCGCTTTTGCTCTC3'		72℃ for 1 min (35 cycles); 72℃ for 10 min

Table 2 Comparison between the results of biopsy-based tests and Stool-PCR

n /Status	Culture	RUT	Histology	Stool-PCR
1/negative	Negative	Nd	Negative	Negative[a]
2/negative	Negative	Nd	Negative	Negative[a]
3/negative	Negative	Nd	Negative	Negative[a]
4/negative	Negative	Negative	Negative	Negative[a]
5/negative	Negative	Negative	Negative	Negative[a]
6/positive	Positive	Negative	Negative	Negative[b]
7/negative	Negative	Negative	Negative	Negative[a]
8/positive	Negative	Positive	Positive	Positive[c]
9/negative	Negative	Positive	Negative	Negative[a]
10/negative	Negative	Negative	Negative	Negative[a]
11/positive	Positive	Positive	Negative	Negative[b]
12/positive	Positive	Positive	Positive	Positive[c]
13/positive	Positive	Positive	Negative	Negative[b]
14/positive	Negative	Positive	Positive	Positive[c]
15/positive	Positive	Positive	Positive	Positive[c]
16/positive	Positive	Positive	Positive	Positive[c]
17/positive	Positive	Positive	Positive	Positive[c]
18/positive	Positive	Positive	Positive	Positive[c]
19/negative	Negative	Negative	Negative	Negative[a]
20/positive	Negative	Positive	Positive	Positive[c]
21/negative	Negative	Negative	Negative	Negative[a]
22/negative	Negative	Negative	Negative	Negative[a]
23/positive	Positive	Positive	Negative	Negative[b]
24/positive	Positive	Positive	Negative	Negative[b]
25/positive	Positive	Positive	Negative	Positive[c]
26/positive	Negative	Positive	Positive	Positive[c]
27/negative	Negative	Positive	Negative	Positive[d]
28/positive	Negative	Positive	Positive	Negative[b]

Nd: Not-determined; a: True negative; b: False negative; c: True positive; d: False positive. Sensitivity: c/c + b = 62.5; Specificity: a/a + d = 92.3%.

Table 3 Comparison of detected genes in DNA from isolates and DNA from stool

n /Status	Detected genes in					
	Isolate			Stool		
	16sRNA	*vacA*	*cagA*	*16sRNA*	*vacA*	*cagA*
1/negative	-	-	-	-	-	-
2/negative	-	-	-	-	-	-
3/negative	-	-	-	-	-	-
4/negative	-	-	-	-	-	-
5/negative	-	-	-	-	-	-
6/positive[a]	+	+	-	-	-	-
7/negative	-	-	-	-	-	-
8/positive[b,c]	-	-	-	-	-	+
9/negative[b]	-	-	-	-	-	-
10/negative	-	-	-	-	-	-
11/positive[a,b]	-	+	+	-	-	-
12/positive[a,b,c]	-	-	+	-	+	-
13/positive[a,b]	+	-	-	-	-	-
14/positive[b,c]	-	-	-	-	+	-
15/positive[a,b,c]	-	+	-	-	+	-
16/positive[a,b,c]	+	+	+	+	+	-
17/positive[a,b,c]	-	-	+	+	+	-
18/positive[a,b,c]	-	-	+	+	+	-
19/negative	-	-	-	-	-	-
20/positive[b,c]	-	-	-	+	+	-
21/negative	-	-	-	-	-	-
22/negative	-	-	-	-	-	-
23/positive[a,b]	-	-	+	-	-	-
24/positive[a,b]	-	-	+	-	-	-
25/positive[a,b]	-	-	+	-	+	-
26/positive[b,c]	-	-	-	-	+	-
27/negative[b]	-	-	-	+	-	-
28/positive[b,c]	-	-	-	-	-	-

a: Culture positive; b: RUT positive; c: Histology positive.

specimens from *H pylori*-negative patients), even after purification procedure. Eleven biopsied children showed positive stool-PCR of which 10 were positive by biopsy-based tests (Table 2). Sensitivity and specificity of stool-PCR were 62.5% and 92.3% respectively.

In this work, detection of *H pylori* specific virulence genes in both isolates and stool (Table 3) was compared. Also, association between endoscopic features, pathology, score of bacteria, and a positive stool-PCR was studied (Table 4). *H pylori* was observed in histological sections of 10 out of 11 stool-positive patients and association was observed between higher score of *H pylori* in histology and a positive stool-PCR.

DISCUSSION

In our previous study[12], we successfully cultured *H pylori* from stool; however, the sensitivity of stool-culture was low. Using PCR, we detected *H pylori* specific genes in isolates and stool in sick and healthy children. However, when fecal extracts were not subjected to column chromatography, there were no results even for the positive controls. This suggests that the method of DNA extraction used in this work efficiently removed the PCR inhibitors. Various methods has been used for the removing of inhibitors or for the purification of DNA

Table 4 Relationship between endoscopic features of patients, histopathology, score of *H pylori* and detection of DNA in stool

n/Status	Endoscopic feature	Histopathology	Score of *H pylori*	Stool PCR
1/negative	Non-ulcer	NSPC	0	Negative
2/negative	Non-ulcer	NST	0	Negative
3/negative	Non-ulcer	Mild chronic gastritis	0	Negative
4/negative	Non-ulcer	Follicular gastritis	0	Negative
5/negative	Non-ulcer	Follicular gastritis + activity	0	Negative
6/positive	Non-ulcer	NSPC	0	Negative
7/negative	Non-ulcer	Mild chronic gastritis	0	Negative
8/positive	Non-ulcer	Follicular gastritis	4	Positive
9/negative	Ulcer	NST	0	Negative
10/negative	Non-ulcer	NSPC	0	Negative
11/positive	Non-ulcer	Follicular gastritis	0	Negative
12/positive	Non-ulcer	Follicular gastritis + activity	4	Positive
13/positive	Non-ulcer	Follicular gastritis	0	Negative
14/positive	Non-ulcer	Moderate chronic gastritis	1	Positive
15/positive	Multiple ulcers	Moderate chronic gastritis	4	Positive
16/positive	Non-ulcer	Moderate chronic gastritis	2	Positive
17/positive	Ulcer	Grading was not possible	1	Positive
18/positive	Non-ulcer	Follicular gastritis + activity	5	Positive
19/positive	Non-ulcer	Mild chronic gastritis	0	Negative
20/positive	Non-ulcer	Follicular gastritis	3	Positive
21/negative	Non-ulcer	NSPC	0	Negative
22/negative	Non-ulcer	NSPC	0	Negative
23/positive	Non-ulcer	Moderate chronic gastritis	0	Negative
24/positive	Non-ulcer	Mild chronic gastritis	0	Negative
25/positive	Non-ulcer	Mild chronic gastritis	0	Positive
26/positive	Non-ulcer	Follicular gastritis	2	Positive
27/negative	Multiple ulcers	Mild chronic gastritis	0	Positive
28/positive	Non-ulcer	Moderate chronic gastritis	3	Negative

NSPC: No significant pathologic change; NST: No suitable tissue.

such as the removal of PCR inhibitors by a polypropylene filter, dilution of fecal suspension, and DNA purification by various biochemical techniques; in many studies with filtration of stool and column chromatography, high sensitivity was observed[8,10-11,14,22-24].

In this work, by detection of various *H pylori* specific genes in stools, 62.5% sensitivity and 92.3% specificity was observed for stool-PCR (Table 2). Nevertheless, by PCR only one or two out of three *H pylori* specific genes were detectable (Table 3). While this permits us to think that the absence of amplification is related to the absence of the detecting gene from the genome or the absence of intact template DNA (in stool), it would be a premature conclusion, since PCR-based absence of an ORF does not necessarily mean its absence from the genome. Also, in a highly recombining genome like *H pylori*, PCR primer annealing sites can pose problems and amplifications may not be generated[25,26]. Thus, we think that for genotyping of *H pylori* from stool, using more than one primer for each gene may enhance detection rate. Many investigators have proposed semi-nested or nested PCR as more sensitive methods for stool-PCR[8,10]. Although these methods reduce background, their disadvantages would be presence of false positive results due to detection of dead bacteria in stool even in low amounts. Sensitivity and specificity of stool-PCR method in this work were acceptable, suggesting that PCR method used in this work was quite adequate for this evaluation.

H pylori is not an intestinal pathogen, and therefore is expected to be present in low concentrations in stool; however, the status of the infection of *H pylori* may influence its density in stool. Thus, we compared histological scoring of *H pylori* with pathological grading and also with the results of stool-PCR. Concordance was observed between higher score of *H pylori* in histological sections and a positive stool-PCR (Table 4). Also, association was observed between the more severe form of gastritis and a positive stool-PCR. Therefore, the degree of stomach colonization by *H pylori* may be important for successful detection of DNA in stool samples. Otherwise, the amount of bacteria excreted in stool may reveal information on the status of *H pylori* infection. Consequently, the association between a higher score of *H pylori* in histology and a positive stool-PCR make it a very useful test for detection of pediatric *H pylori* infection.

ACKNOWLEDGMENTS

We thank MRS Shahla Tafreshi and MRS Masoumeh Madadi for technical assistance. Mavenat Pajouheshy of Alzahra University, Vanak, Tehran has supported this work.

COMMENTS

Background

A reliable non-invasive test for detection of *Helicobacter pylori* (*H pylori*) infection in routine practice is essential, especially for children since the application of biopsy-based tests is more difficult for them. Serological tests do not necessarily indicate active infection by *H pylori*, and urea breath test (UBT) is expensive and not available in routine clinical laboratories, especially in developing countries. The *H pylori* stool-antigen (HpSA) test has been shown to be very useful, especially in children; however, various commercial tests have shown some discrepancies in different geographical areas. Stool-PCR may be a very useful test in specific detection of *H pylori*. In this study, we evaluated the performance of stool-PCR in diagnosis of active infection in children.

Research frontiers

Stool-PCR is a very useful method for detection of *H pylori* genes in stool. It is interesting because *H pylori* specific genes, including virulence genes and the genes involved in its resistance to antibiotics, can be detected by this method. Furthermore, a positive stool-PCR has significance in relation to the status of stomach colonization by *H pylori*.

Innovations and breakthroughs

A stool-PCR method such that used in this work may represent a very specific test for diagnosis of *H pylori* infection. This is the first study to report association between a positive stool-PCR and the degree of stomach colonization, manifested by higher score of *H pylori* in histology.

Applications

A simple PCR method such that used in this work will be quite adequate for detection of *H pylori* infection.

Peer review

In this study, Falsafi *et al*. evaluated the performance of stool-PCR test for diagnosis of current *H pylori* infection in children. The content of the article can be interesting for gastroenterologists who work with the pediatric population, especially with very young children and patients with certain neurological disorders. Stool-PCR may be a very useful method in detection of *H pylori* infection.

REFERENCES

1 **Fischbach W**, Chan AO, Wong BC. Helicobacter pylori and Gastric Malignancy. *Helicobacter* 2005; **10** Suppl 1: 34-39
2 **Wundisch T**, Thiede C, Morgner A, Dempfle A, Gunther A, Liu H, Ye H, Du MQ, Kim TD, Bayerdorffer E, Stolte M, Neubauer A. Long-term follow-up of gastric MALT lymphoma after Helicobacter pylori eradication. *J Clin Oncol* 2005; **23**: 8018-8024
3 **Elitsur Y**, Yahav J. Helicobacter pylori infection in pediatrics. *Helicobacter* 2005; **10** Suppl 1: 47-53
4 **Kusters JG**, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. *Clin Microbiol Rev* 2006; **19**: 449-490
5 **Kikuchi S**, Dore MP. Epidemiology of Helicobacter pylori Infection. *Helicobacter* 2005; **10** Suppl 1: 1-4
6 **Krogfelt KA**, Lehours P, Megraud F. Diagnosis of Helicobacter pylori Infection. *Helicobacter* 2005; **10** Suppl 1: 5-13
7 **Monteiro L**, de Mascarel A, Sarrasqueta AM, Bergey B, Barberis C, Talby P, Roux D, Shouler L, Goldfain D, Lamouliatte H, Megraud F. Diagnosis of Helicobacter pylori infection: noninvasive methods compared to invasive methods and evaluation of two new tests. *Am J Gastroenterol* 2001; **96**: 353-358
8 **Kabir S**. Detection of Helicobacter pylori in faeces by culture, PCR and enzyme immunoassay. *J Med Microbiol* 2001; **50**: 1021-1029
9 **Roth DE**, Taylor DN, Gilman RH, Meza R, Katz U, Bautista C, Cabrera L, Velapatino B, Lebron C, Razuri M, Watanabe J, Monath T. Posttreatment follow-up of Helicobacter pylori infection using a stool antigen immunoassay. *Clin Diagn Lab Immunol* 2001; **8**: 718-723
10 **Makristathis A**, Pasching E, Schutze K, Wimmer M, Rotter ML, Hirschl AM. Detection of Helicobacter pylori in stool specimens by PCR and antigen enzyme immunoassay. *J Clin Microbiol* 1998; **36**: 2772-2774
11 **Monteiro L**, Gras N, Megraud F. Magnetic immuno-PCR assay with inhibitor removal for direct detection of Helicobacter pylori in human feces. *J Clin Microbiol* 2001; **39**: 3778-3780
12 **Falsafi T**, Valizadeh N, Najafi M, Ehsani A, Khani A, Landarani Z, Falahi Z. Culture of Helicobacter pylori from stool samples in children. *Can J Microbiol* 2007; **53**: 411-416
13 **Li YH**, Guo H, Zhang PB, Zhao XY, Da SP. Clinical value of Helicobacter pylori stool antigen test, ImmunoCard STAT HpSA, for detecting H pylori infection. *World J Gastroenterol* 2004; **10**: 913-914
14 **Makristathis A**, Barousch W, Pasching E, Binder C, Kuderna C, Apfalter P, Rotter ML, Hirschl AM. Two enzyme immunoassays and PCR for detection of Helicobacter pylori in stool specimens from pediatric patients before and after eradication therapy. *J Clin Microbiol* 2000; **38**: 3710-3714
15 **Manes G**, Balzano A, Iaquinto G, Ricci C, Piccirillo MM, Giardullo N, Todisco A, Lioniello M, Vaira D. Accuracy of the stool antigen test in the diagnosis of Helicobacter pylori infection before treatment and in patients on omeprazole therapy. *Aliment Pharmacol Ther* 2001; **15**: 73-79
16 **Delgado S**, Suarez A, Otero L, Mayo B. Variation of microbiological and biochemical parameters in the faeces of two healthy people over a 15 day period. *Eur J Nutr* 2004; **43**: 375-380
17 **van Tongeren SP**, Slaets JP, Harmsen HJ, Welling GW. Fecal microbiota composition and frailty. *Appl Environ Microbiol* 2005; **71**: 6438-6442
18 **Falsafi T**, Valizadeh N, Sepehr S, Najafi M. Application of a stool antigen test to evaluate the incidence of Helicobacter pylori infection in children and adolescents from Tehran, Iran. *Clin Diagn Lab Immunol* 2005; **12**: 1094-1097
19 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181
20 **Zaitoun AM**. The prevalence of lymphoid follicles in Helicobacter pylori associated gastritis in patients with ulcers and non-ulcer dyspepsia. *J Clin Pathol* 1995; **48**: 325-329
21 **Park CY**, Kwak M, Gutierrez O, Graham DY, Yamaoka Y. Comparison of genotyping Helicobacter pylori directly from biopsy specimens and genotyping from bacterial cultures. *J Clin Microbiol* 2003; **41**: 3336-3338
22 **MacKay WG**, Williams CL, McMillan M, Ndip RN, Shepherd AJ, Weaver LT. Evaluation of protocol using gene capture and PCR for detection of Helicobacter pylori DNA in feces. *J Clin Microbiol* 2003; **41**: 4589-4593
23 **Sen N**, Yilmaz O, Simsek I, Kupelioglu AA, Ellidokuz H. Detection of Helicobacter pylori DNA by a simple stool PCR method in adult dyspeptic patients. *Helicobacter* 2005; **10**: 353-359
24 **Liang S**, Redlinger T. A protocol for isolating putative Helicobacter pylori from fecal specimens and genotyping using vacA alleles. *Helicobacter* 2003; **8**: 561-567
25 **Hanage WP**, Fraser C, Spratt BG. The impact of homologous recombination on the generation of diversity in bacteria. *J Theor Biol* 2006; **239**: 210-219
26 **Kraft C**, Suerbaum S. Mutation and recombination in Helicobacter pylori: mechanisms and role in generating strain diversity. *Int J Med Microbiol* 2005; **295**: 299-305

S- Editor Tian L **L- Editor** Li M **E- Editor** Ma WH