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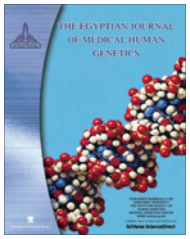

ORIGINAL ARTICLE

Salivary PCR detection of *Helicobacter pylori* DNA in Egyptian patients with dyspepsia

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Abstract Several methods are available for detecting *Helicobacter pylori* infection: (1) invasive methods based on gastric biopsies, (2) non invasive methods like Urea Breath Test (UBT), serology and stool antigen tests. Importance of salivary PCR in detection of *H. pylori* is still questionable. To evaluate the role of salivary PCR technique in detecting *H. pylori* gastric affection in Egyptian patients with dyspepsia and in differentiating between functional dyspepsia and acid-ulcer syndrome. This study included 60 patients with dyspepsia classified into three groups: (Group 1) patients with gastric *H. pylori* and ulcers or erosions ($n = 20$), (Group 2) patients with gastric *H. pylori* and no ulcers or erosions and had functional dyspepsia ($n = 20$), (Group 3) patients without *H. pylori* and had functional dyspepsia ($n = 20$). All underwent upper gastrointestinal endoscopy with biopsies, rapid urease test and salivary samples for *H. pylori* PCR. Significant difference between the three groups regarding salivary PCR values. No significant difference between Group 1 and Group 2 but both had significant difference with Group 3, significant difference between gastric *H. pylori* positive patients ($n = 40$) and negative ones ($n = 20$). Salivary PCR test had sensitivity of 85%, specificity of 70% in diagnosing *H. pylori*. PCR value of 534000 Iu/ml had best sensitivity (75%) and specificity (100%) for diagnosing *H. pylori*, highly significant positive correlation

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between *H. pylori* gastric affection and salivary PCR values. No significant difference between patients with acid ulcer syndrome ($n = 20$) and those with functional dyspepsia ($n = 40$) as regard salivary PCR mean values. Salivary PCR test showed sensitivity of 100%, specificity of 50% in differentiating between patients with acid ulcer syndrome and those with functional dyspepsia. PCR value of 440000 Iu/ml had best sensitivity (100%) and specificity (55%) in differentiating acid ulcer syndrome from functional dyspepsia with non significant. *H. pylori* salivary PCR may be of value in diagnosing *H. pylori* gastric affection and is strongly correlated with it but it is of limited value in differentiating between acid ulcer syndrome and functional dyspepsia.

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1. Introduction

It is estimated that 50% of the world's population is infected by *Helicobacter pylori* [1]. Around the world, the prevalence of *H.* pylori infection ranges from 20% to over 90% in adult populations. Infection rates average at about 30% in Western populations while infection rates in Asian countries and in developing countries are higher and range from 60% to 90% [2]. According to Mohammad et al. [3], the overall prevalence of *H. pylori* infection was 72.38% among Egyptian school children.

H. pylori infection is associated with chronic gastritis, peptic ulcers, atrophic gastritis, intestinal metaplasia, gastric adenomas, gastric hyperplastic polyps, adenocarcinomas of the distal part of the stomach, and lymphomas of mucosa-associated lymphoid tissue [4]. These diseases, in most instances, develop many years after the host colonization [5]. The World Health Organization has categorized *H.* pylori infection as a definite human carcinogen class I since 1994 [6].

H. pylori infection can be diagnosed by invasive techniques requiring endoscopy and biopsy (histological examination, culture, polymerase chain reaction) and by noninvasive techniques (serology, urea breath test, detection of *H. pylori* antigen in stool specimen) [7].

Human is the only known host of *H.* pylori. Its transmission route is not yet clearly understood. Epidemiological studies suggest person-to-person transmission, by either fecal–oral or oral–oral routes, to be the major mechanism. In developing countries, there is evidence for both food- and water-borne transmission of *H.* pylori [8]. The human stomach is considered as the reservoir of this pathogen [9].

The first documentation of the presence of *H. pylori* in the oral cavity was reported in 1989, when the bacterium was cultured from the dental plaque of one of 29 patients with *H.* pylori associated gastric disease [10]. Since then, some reports indicated that Helicobacter may be present in oral cavity (particularly gingival pockets) which can serve as a reservoir for bacteria and a source of gastric reinfection [11]. *H. pylori* has also been detected by culture and PCR in both dental plaques and saliva [12].

In 2006, ''Rome III'' classification [13] functional dyspepsia (FD) was included in the subcategory of functional gastroduodenal disorders [14]. Of patients with functional dyspepsia, 30–60% carry *H. pylori*, but this prevalence is not much different from that in the unaffected population [15]. Various studies have focused on the effect of *H.* pylori eradication in patients with both functional and uninvestigated dyspepsia. A meta-analysis of 13 randomized studies of functional (non-ulcer) dyspepsia showed that *H.* pylori eradication was associated with an 8% relative risk reduction compared with placebo [16].

2. Aim of the work

This study aimed to evaluate the role of salivary PCR technique in detecting *H.* pylori gastric affection in Egyptian patients with dyspepsia and in differentiating between functional dyspepsia and acid-ulcer syndrome.

3. Patients and methods

In this study 60 dyspeptic patients (defined symptomatically as abdominal discomfort related to meal) attending the outpatient clinics of Internal Medicine and Tropical Medicine Departments, Ain Shams University Hospital were included after approval of the ethical committee and the taking of their informed consent.

All patients with hepatic, pulmonary, renal and cardiac diseases or with contraindication to endoscopy were excluded from the study. All studied patients did not receive non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPI) or antibiotics within the previous two months.

All studied patients were subjected to: History taking, Complete clinical examination, Complete blood picture, Kidney function tests (urea and creatinine), Liver function tests (SGOT, SGPT, serum bilirubin, serum albumin, prothrombin time). Upper GIT endoscopy by the same operator: to evaluate for the presence of gastritis, erosions or ulcers. Four quadrant biopsies were taken from antral mucosa within 5 cm of the pyloric opening for detection of *H. pylori* infection using [(1) rapid urease test (2) Histopathological examination] and Salivary PCR for *H. pylori DNA*.

- Rapid urease test: One biopsy specimen was placed in urea broth, incubated at 37 °C, and examined after 4 hours and after overnight incubation. Urease positive test changed the color of the indicator from yellow to purple – pink [27].
- Histopathological examination: Biopsy taken from antral mucosa was fixed in 10% buffer formalin and was prepared from paraffin block for modified Giemsa stain for detection of *H. pylori* [27].
- Salivary PCR for *H. pylori* DNA examination: Saliva samples (2–3 ml) were collected in a sterile container prior to endoscopy. DNA extraction was done with MagNA pure compact Nucleic Acid Isolation Kit (Roche Diagnostics Nederland BV) according to the manufacturer's instructions. Real-time PCR was performed with a Light Cycler (Roche, Mannheim, Germany). for Real-Time PCR analysis, The nucleic acid sequence for the oligonucleotide primer pairs specific for two sequences flanking an internal 294 bp fragment of the urease C gene of *H. pylori*, These oligonucleotides

were Forward primer was 5′ AAG CTT TTA GGG TTG TTA GGG GTT T-3′ and for the Reverse primer was 5′ AAG CTT ACT TTC TAA CAC TAA CGC 3′ both were supplied by Tip Mol Biol, USA. Master mix was prepared to reach a total volume of 20 µl: (5.0 µl 10X reaction buffer, 5.0 µl dNTPs 10 mM, primer set final 100 nM each (forward & reverse primers), 0.5 µl Prime Taq™ DNA polymerase (DNA plasmid)(Quality, Genet Bio Roche diagnostic, with Syber Green buffer, Korea), serial dilution of positive standard control 10^7–10^2 copies/ml for calibration curve, 5.0 µl DNA, RNAse-free water). The thermal cycler was programmed in three steps: (Step l: 5 min at 94 °C, Step 2: 30 sec. at 94 °C, 30 sec. at 52° C. 1 min at 72 °C, this was repeated for 35 cycles, Step 3: 5 min at 72 °C) [28].

According to the results of upper GIT endoscopy, *H. pylori* rapid urease and histopathology, selected patients were divided into 3 groups. Group 1: Patients confirmed to be gastric *H. pylori* positive and with ulcers or erosions (20 patients). Group 2: Patients with functional dyspepsia (according to Rome III criteria) confirmed to be gastric *H. pylori* positive without ulcers or erosions (20 patients). Group 3: Patients with functional dyspepsia (according to Rome III criteria) proved to be gastric *H. pylori* negative (20 patients).

3.1. Statistical analysis

Data were collected, revised, verified, and then edited and analyzed statistically using Statistical Package for Social Sciences program (SPSS v16).The following tests were used in this study: mean, standard deviation, T test for independent samples, ANOVA test (analysis of variance), Post Hoc test, Spearman coefficient of rank correlation (rho), ROC curve analysis. Significance levels: $P > 0.05$ insignificant, $P < 0.05$ significant and $P < 0.001$ highly significant.

4. Results

This study included 32 males (53%) and 28 females (47%) presented with dyspepsia (defined as abdominal discomfort related to meals) with their mean age of 35.63 ± 14.23 years.

Patients were divided into three groups:

Group 1: their mean age was 41.4 ± 15.1 years, (60% males and 40% females).

Group 2: their mean age was 36.8 ± 16.7 years, (50% males and 50% females).

Group 3: their mean age was 28.7 ± 7.34 years, (50% males and 50% females).

- Salivary PCR test was positive in 20 /20 patients (100%) of Group 1, 14/20 (70%) of Group 2 and 6/20 (30%) of Group 3.
- On comparing the three groups as regard *H. pylori* salivary PCR values Table 1, we found significant difference between the three groups ($P = 0.04$) with patients with functional dyspepsia and gastric *H. pylori* (Group 2) showed the highest values. Post hoc analysis revealed that there was no significant difference between Group 1 and Group 2 ($P = 0.3018$) but there was significant difference between Group 1 and Group 3 ($P = 0.0056$) (Group 1 mean values were higher than Group 3) and highly significant difference between Group 2 and Group 3 ($P < 0.0001$) (Group 2 mean values were higher than Group 3).
- Comparison between gastric *H. pylori* positive patients ($n = 40$) and gastric *H. pylori* negative patients ($n = 20$) (regarding salivary PCR test):
  1. There was significant difference between both groups ($P = 0.0017$) with gastric *H. pylori* positive patients having higher salivary PCR mean value Table 2.
  2. Among the 40 gastric *H. pylori* positive patients, 34 patients (85%) had positive salivary PCR. While among the 20 gastric *H. pylori* negative ones, only 6 patients (30%) showed positive salivary PCR. This gives the PCR test sensitivity of 85%, specificity of 70%, positive predictive value of 85% and negative predictive value of 70% in diagnosing *H. pylori* affection Table 3.
  3. The ROC curve analysis showed that the best cutoff value for diagnosing *H. pylori* positivity by salivary PCR was 534000 Iu/ml with the best sensitivity (75%) and specificity (100%) and highly significant *P*-value ($P = 0.0001$)
  4. Spearman coefficient of rank correlation showed highly significant positive correlation (rho = 0.649) between *H. pylori* gastric affection and salivary PCR ($P = 0.0001$).
- Comparison between patients with acid ulcer syndrome ($n = 20$) and those with functional dyspepsia ($n = 40$):
  1. There was no significant difference between both groups as regards salivary PCR mean values ($P = 0.598$) (Table 2).
  2. Salivary PCR test was positive in 20/20 patients (100%) with acid ulcer syndrome and in 20/40 patients (50%) with functional dyspepsia. This gives the test a sensitivity of 100%, specificity of 50%, positive predictive value of 50% and negative predictive value of 100% in differentiating between patients with acid ulcer syndrome and those with functional dyspepsia (Table 3).
  3. The ROC curve analysis showed that the best cutoff value for differentiating acid ulcer syndrome from functional dyspepsia by salivary PCR was 440000 Iu/ml with the best sensitivity (100%) and specificity (55%) but with non significant *P*-value ($P = 0.0508$) (Fig. 1).

Table 1 Comparison between the three groups as regard salivary PCR values.

Salivary PCR	Group 1 ($n = 20$)	Group 2 ($n = 20$)	Group 3 ($n = 20$)
Mean	948300	1378800	144500
SD	±388252.22	±1220199	±233760.20
F	6.950		
P	0.04 (S)		

Table 2 Comparison between gastric *H. pylori* positive and negative patients and comparison between patients with acid ulcer syndrome and those with functional dyspepsia (regarding salivary PCR test).

Salivary PCR	Gastric *H. pylori* positive ($n = 40$)	Gastric *H. pylori* negative ($n = 20$)	Acid ulcer syndrome ($n = 20$)	Functional dyspepsia ($n = 40$)
Mean	1163550	144500	948300	761650
SD	±908534.9384	±233760.2048	±388252.2233	±1063985.7179
T-test	−3.462		−0.533	
P	0.0017 (S)		0.5980 (NS)	

Table 3 Salivary PCR test in diagnosing *H. pylori* affection and in differentiating between acid ulcer syndrome and functional dyspepsia.

Parameter	Gastric *H. pylori* positive ($n = 40$)	Gastric *H. pylori* negative ($n = 20$)	Acid ulcer syndrome ($n = 20$)	Functional dyspepsia ($n = 40$)
Salivary PCR positive	34	6	20	20
Salivary PCR negative	6	14	0	20
Sensitivity = 85%, specificity = 70%, positive predictive value = 85% negative predictive value = 70%			Sensitivity = 100% specificity = 50% positive predictive value = 50% negative predictive value = 100%	

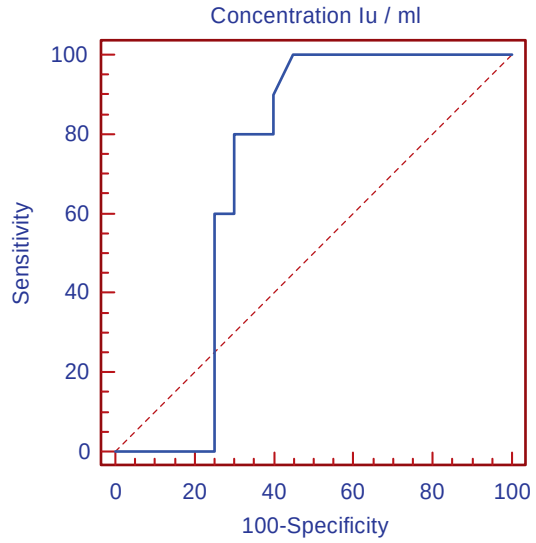

Figure 1 Salivary PCR ROC curve for differentiating between acid ulcer syndrome and functional dyspepsia patients.

5. Discussion

Recent studies have demonstrated that *H. pylori* can be found in the human oral cavity as it provides an excellent microaerophilic environment, therefore, being a potential reservoir for *H. pylori*. It is unclear whether the oral cavity is a permanent or transient reservoir [12,17,18]. Souto and Colombo [19] found that 20% and 33% of subjects had positive samples in saliva and dental plaque, respectively.

One of the first investigations of the influence of oral *H. pylori* on stomach infection was carried out by Miyabayashi et al. [20]. Their study revealed that there is relationship between gastritis induced by *H. pylori* infection and oral colonization of the bacterium and that oral *H. pylori* is resistant to typical triple anti-*H. pylori* therapy used to eradicate it from the stomach. So, patients with oral *H. pylori* were at a significantly greater risk of gastric reinfection following successful therapy.

Both cultures and urease tests carried out on the oral cavity samples revealed that the methodology used for the detection of *H. pylori* is not sufficiently sensitive for the detection of the microorganism in the oral cavity [22,23]. Successful amplification and specific detection of *H. pylori* DNA directly from salivary samples in the majority of infected subjects indicates that this approach is feasible and demonstrates that it has true potential in aiding the diagnosis and management of patients with active *H. pylori* infection [21]. But the results of salivary PCR are still conflicting and differ among various studies.

Rasmussen et al. [24] studied 78 adults presenting with recurrent abdominal pain. *H. pylori* infection was confirmed from gastric biopsies using PCR, Southern blotting, histology and urease test and compared the results with salivary PCR technique results. Of the 66 patients who were *H. pylori* positive in their gastric biopsies, salivary PCR was positive in 71.2%. Salivary PCR was positive in 50% of gastric *H. pylori* negative patients. A statistically significant correlation was observed between the presence of *H. pylori* in the gastric biopsies and the oral cavity ($P < 0.0001$).

Tiwari et al. [21] studied one hundred patients (80 symptomatic with dyspepsia and 20 asymptomatic) who underwent gastroscopy and were investigated for the presence of *H. pylori* in saliva and stomach. Seventy two of the symptomatic group and ten asymptomatic subjects were infected with *H. pylori* in the stomach as determined by histology and direct PCR amplification of gastric biopsy DNA obtained from each subject. *H. pylori* DNA was identified in the saliva of seventy symptomatic subjects and twelve asymptomatic control subjects.

In the study performed by Silva et al. [25], *H. pylori* was not detected in saliva in any of the control group (individuals with no gastric disease who were *H. pylori* positive). In the case

group, (individuals with gastric disease who were *H. pylori* positive) *H. pylori* DNA was detected in 16/30 (53.3%) saliva samples.

In the current study, there was significant difference ($P = 0.0017$) between group of patients with gastric *H. pylori* infection ($n = 40$) and those without ($n = 20$) as regard salivary PCR values. Patients having gastric *H. pylori* infection showed higher salivary PCR mean values (1163,550 vs. 144,500, respectively). Thirty four out of the forty gastric *H. pylori* positive patients (85%) had positive salivary PCR. On the other hand, 6 out of the 20 gastric *H. pylori* negative patients (30%) were *H. pylori* positive by salivary PCR. Our results showed lower detection rates than the results of Tiwari et al. [21].On the contrary, this study results showed higher detection rates than those reported by Silva et al. [25] and Rasmussen et al. [24]. This study is not in agreement with the study of Kignel et al. [26] who found that of the 20 patients with *H. pylori* detected by rapid urease in the stomach, the organism was detected in none of the salivary samples by PCR (0%).

The discrepancies in the detection rates of *H. pylori* by salivary PCR test that were found between the current study and other studies may be attributed to factors affecting PCR accuracy. These factors include the choice of primers and target DNA, specimen preparation, bacterial density, and technical issues regarding the PCR procedure [29]. Previous investigators stated that because *H. pylori* in saliva generally reflects the reflux of organisms from the stomach, their detection rates may vary. There is also the possibility of cross-reactivity with spiral urease-containing organisms normally present in the mouth, especially if primer pairs are not carefully selected [30].

Our results showed highly significant positive correlation between *H. pylori* gastric affection and salivary PCR values (rho = 0.649, $P = 0.0001$). This is consistent with the results of Rasmussen et al. [24]. On evaluating the role of salivary PCR in *H. pylori* gastric detection, salivary PCR test had a sensitivity of 85%, specificity of 70%, positive predictive value of 85% and negative predictive value of 70% making this test valuable in diagnosing *H. pylori* gastric affection. Using ROC curve analysis, salivary PCR test showed best sensitivity (75%) and specificity (100%) for diagnosing *H. pylori* gastric positivity at cutoff value of 534,000 Iu/ml with highly significant *P*-value ($P = 0.0001$).

PCR values were higher in patients with functional dyspepsia and gastric *H.* pylori (group2) followed by patients with ulcers or erosions and *H.* pylori (group 1) then patients with functional dyspepsia who are gastric *H. pylori* negative (group 3). There was no significant difference between Group 1 and Group 2, but there was significant difference between each group and group 3.

On evaluating the role of salivary PCR test as a tool in differentiating acid ulcer syndrome patients ($n = 20$) from functional dyspepsia patients ($n = 40$), we found no significant difference between both groups as regard salivary PCR mean values ($P = 0.598$) and salivary PCR test was not of value making differentiation between both groups.

6. Conclusion

Helicobacter pylori salivary PCR may be of value in diagnosing *H. pylori* gastric affection and is strongly correlated with it but it is of limited value in differentiating between acid ulcer syndrome and functional dyspepsia.

References

[1] Torres J, Pérez-Pérez G, Goodman K, Atherton J, Gold B, Harris P, et al. A comprehensive review of the natural history of Helicobacter pylori infection in children. Arch Med Res 2000;31:431–69.

[2] Prinz C, Schwendy S, Voland P. H pylori and gastric cancer: shifting the global burden. World J Gastroenterol 2005;12:5458–64.

[3] Mohammad M, Hussein L, Coward A, Jackson S. Prevalence of Helicobacter pylori infection among Egyptian children: impact of social background and effect on growth. Public Health Nutr 2008;11(3):230–6.

[4] Everhart JE. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin North Am 2000;29:559–78.

[5] Smith VC, Genta R M. Role of Helicobacter pylori gastritis in gastric atrophy, intestinal metaplasia, and gastric neoplasia. Microsc Res Tech 2000;48:313–20.

[6] Ouakaa-Kchaou A, Elloumi H, Gargouri D, Kharrat J, Ghorbel A. Helicobacter pylori and gastric cancer. Tunis Med 2010;88(7):459–61.

[7] Vaira D, Gatta L, Ricci C, Miglioli M. Review article: diagnosis of Helicobacter pylori infection. Aliment Pharmacol Ther 2002;16(1):16–23.

[8] Mendall MA. Transmission of Helicobacter pylori. Semin Gastrointest Dis 1997;8:113–23.

[9] Das J, Paul N. Epidemiology and pathophysiology of Helicobacter pylori infection in children. Ind J Pediatr 2007;74:287–90.

[10] Krajden S, Fuksa M, Anderson J, Kempston J, Boccia A, Petrea C, et al. Examination of human stomach biopsies, saliva, and dental plaque for Campylobacter pylori. J Clin Microbiol 1989;27:1397–8.

[11] Karczewska E, Konturek J, Konturek P, Cześnikiewicz M, Sito E, Bielański W. Oral cavity as a potential source of gastric reinfection by Helicobacter pylori. Dig Dis Sci 2002;47(5):978–86.

[12] Dowsett SA, Kowolik MJ. Oral Helicobacter pylori: can we stomach it? Crit Rev Oral Biol Med 2003;14:226–33.

[13] Drossman DA. The functional gastrointestinal disorders and the Rome III process. Gastroenterology 2006;130:1377–90.

[14] Tack J, Talley N, Camilleri M, Holtmann G, Hu P, Malagelada J, et al. Functional gastroduodenal disorders. Gastroenterology 2006;130:1466–79.

[15] Talley NJ, Hunt RH. What role does Helicobacter pylori play in dyspepsia and nonulcer dyspepsia? Arguments for and against *H. pylori* being associated with dyspeptic symptoms. Gastroenterology 1997;113:67–77.

[16] Moayyedi P, Soo S, Deeks J, Delaney B, Harris A, Innes M, et al. Eradication of Helicobacter pylori for nonulcer dyspepsia. Cochrane Database Syst Rev 2005;25(1), CD002096.

[17] Loster B, Majewski S, Cze[nikiewicz-Guzik M, Bielanski W, Pierzchalski P, Konturek S. The relationship between the presence of Helicobacter pylori in the oral cavity and gastritis in the stomach. J Physiol Pharmacol 2006;57:91–100.

[18] Bürgers R, Schneider-Brachert W, Reischl U, Behr A, Hiller K, Lehn N, et al. Helicobacter pylori in human oral cavity and stomach. Eur J Oral Sci 2008;116:297–304.

[19] Souto R, Colombo A. Detection of Helicobacter pylori by polymerase chain reaction in the subgingival biofilm and saliva of non-dyspeptic periodontal patients. J Periodontol 2008;79:97–103.

[20] Miyabayashi H, Furihata K, Shimizu T, Ueno I, Akamatsu T. Influence of oral Helicobacter pylori on the success of eradication

therapy against gastric Helicobacter pylori. Helicobacter 2000;5: 30–7.

[21] Tiwari S, Khan A, Ahmed K, Ahmed I, Kauser F, Hussain M, et al. Rapid diagnosis of Helicobacter pylori infection in dyspeptic patients using salivary secretion: a non-invasive approach. Singapore Med J 2005;46(5):224–8.

[22] Li C, Ha T, Ferguson D, Chi D, Zhao R, Patel N, et al. A newly developed PCR assay of *H. pylori* in gastric biopsy, saliva and feces. Evidence of high prevalence of *H. pylori* in saliva supports oral transmission. Dig Dis Sci 1996;41:2142–9.

[23] De Sousa L, Vásquez L, Velasco J, Parlapiano D. Isolation of Helicobacter pylori in gastric mucosa, dental plaque and saliva in a population from the Venezuelan Andes. Invest Clin 2006;47(2):109–16.

[24] Rasmussen LT, Labio RW, Gatti LL, Silva LC, Queiroz VF, Smith MA, Payão SL. Helicobacter pylori detection in gastric biopsies, saliva and dental plaque of Brazilian dyspeptic patients. Mem Inst Oswaldo Cruz 2010;105(3):326–30.

[25] Silva DG, Stevens RH, Macedo JM, Albano RM, Falabella ME, Veerman EC, Tinoco EM. Detection of cytotoxin genotypes of Helicobacter pylori in stomach, saliva and dental plaque. Arch Oral Biol 2009;54:684–8.

[26] Kignel S, de Almeida Pina F, André EA, Alves Mayer MP, Birman EG. Occurrence of Helicobacter pylori in dental plaque and saliva of dyspeptic patients. Oral Dis 2005;11(1): 17–21.

[27] Logan RP, Walker MM. ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of Helicobacter pylori infection. BMJ 2001;323(7318):920–2.

[28] Mapstone NP. The detection of Helicobacter pylori by the polymerase chain reaction. In: Clayton CL, Mobley HLT, editors. Methods in Molecular Medicine: Helicobacter pylori Protocols. Totowa, NJ: Humana Press Inc; 1997. p. 31–6.

[29] Dunn B, Cohen H, Blaser M. Helicobacter pylori. Clin Microbiol Rev 1997;10(4):720–41.

[30] Sugimoto M, Wu J, Abudayyeh S, Hoffman J, Brahem H, Al-Khatib K, Yamaoka Y, Graham D. Unreliability of Results of PCR Detection of *Helicobacter pylori* in Clinical or Environmental Samples. J Clin Microbiol 2009;47(3):738–42.