Oral Microbiology Immunology 2009: 24: 464–468
Printed in Singapore. All rights reserved

ORAL MICROBIOLOGY AND IMMUNOLOGY

Helicobacter pylori in the oral cavity is associated with gastroesophageal disease

R. Morales-Espinosa[1], A. Fernandez-Presas[1], G. Gonzalez-Valencia[2], S. Flores-Hernandez[3], G. Delgado-Sapien[1], J. L. Mendez-Sanchez[1], E. Sanchez-Quezada[1], L. Muñoz-Pérez[2], R. Leon-Aguilar[4], J. Hernandez-Guerrero[4], A. Cravioto[1]

[1]Laboratory of Bacterial Genomics, Department of Microbiology and Parasitology, Faculty of Medicine, Universidad Nacional Autónoma de México, Mexico City, Mexico, [2]Unit of Medical Investigation in Infectious and Parasitological Diseases, Mexican Institute of Social Security (IMSS), XXI Century, Mexico City, Mexico, [3]National institute of Respiratory Diseases, Secretary of Health, Mexico City, Mexico, [4]Faculty of Odontology, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico

Morales-Espinosa R, Fernandez-Presas A, Gonzalez-Valencia G, Flores-Hernandez S, Delgado-Sapien G, Mendez-Sanchez JL, Sanchez-Quezada E, Muñoz-Pérez L, Leon-Aguilar R, Hernandez-Guerrero J, Cravioto A. Helicobacter pylori *in the oral cavity is associated with gastroesophageal disease*.
Oral Microbiol Immunol 2009: 24: 464–468. © 2009 John Wiley & Sons A/S.

Background: In Mexico, more than 80% of the population is infected with *Helicobacter pylori*. The frequency of *H. pylori* detection in the oral cavity is unknown, as its relationship with gastroesophageal pathology.
Aim: To detect the presence of *H. pylori* in the oral cavity in Mexican population by PCR and to determine its association with gastroesophageal disease.
Methods: Patients were divided into two groups with different clinic conditions from whom gastric biopsy, dental plaque, and saliva samples were taken and analyzed. The first group comprised of hospitalized patients, the majority of whom were diagnosed with gastroesophageal disease, while the second group was selected from a dental clinic (ambulatory population) the majority of whom appeared to be healthy subjects.
Results: *H. pylori* was detected in gastric biopsy, dental plaque and saliva samples by PCR using a set of specific primers for the signal sequence of the vacuolating cytotoxin gene; detection of *H. pylori* in general was higher in gastric biopsy and dental plaque samples than in saliva samples. Detection of *H. pylori* in the oral cavity is significantly ($P = 0.0001$) associated with patients presenting gastroesophageal disease, while healthy subjects and those with other non-gastric disease do not present with *H. pylori* in their oral cavity.
Conclusions: *H. pylori* detection in the oral cavity is associated to gastroesophageal disease. In addition, it is suggested that all patients presenting gastric symptoms and *H. pylori* detection in the oral cavity would begin bacterial treatment immediately.

Key words: *Helicobacter pylori*; oral cavity

Rosario Morales-Espinosa, Laboratory of Bacterial Genomics, Department of Microbiology and Parasitology, Faculty of Medicine, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Colonia Copilco, Delegación Coyoacan, C.P. 04510, Mexico City, Mexico
Tel.: +525 5523 2135;
fax: +525 5623 2114;
e-mail: marosari@servidor.unam.mx

Accepted for publication June 22, 2009

Helicobacter pylori is a pathogen that causes chronic gastritis, gastric and duodenal ulceration, and in a few cases, contributes to the development of gastric carcinoma and Mucosa-Associated Lymphoid Tissue (MALT) lymphoma (2, 7). The detection and isolation of bacteria from the gastric mucosa has been performed successfully (1, 6, 12, 19), however, reports of extra-gastric isolation, such as from the oral cavity, are still controversial (5, 9, 11, 13, 17, 18). Various authors have suggested that *H. pylori* detection using PCR protocols in the oral cavity might support the assumption that the mouth is a reservoir for re-infection of the gastric cavity by this microorganism, (8, 22), although this suggestion remains controversial. On the other hand, some studies have speculated that *H. pylori* may be a commensal organism and its presence does not mean that infection will occur in the stomach (24). Additionally, other researchers have suggested that *H. pylori* sometimes colonizes dental plaque but that such colonization appears to be transient as the microorganism passes through the oral cavity to the stomach (20). The truth is that the number of *H. pylori* organisms necessary to cause infection or disease in the stomach is unknown. In addition, it is not clear if the presence of the bacteria in the mouth is transient or not, if additional risk factors exist that favor *H. pylori* growth in the oral cavity, or if there is a number of sufficient microorganisms in dental plaque

that could serve as a reservoir for re-infection of the gastric mucosa in susceptible patients, following previous eradication of gastric infection by antibiotics. A number of studies using PCR have been carried out in various parts of the world that have also shown contradictory results in terms of *H. pylori* detection frequency in the oral cavity with a range between 0 and 100% (9, 11, 13, 15, 17, 23). This wide variation is probably due to the type of population studied, and the differences in specificity and sensitivity of the primers used in the PCR tests. In Mexico, the seroprevalence of *H. pylori* infection is more than 80% (28), and co-infection with multiple *H. pylori* strains is common (12, 19). However, the frequency of *H. pylori* colonization in the oral cavity is unknown, as is the relationship between its detection and gastric pathology. Using a simple PCR methodology, the aim of this study was to determine the presence of *H. pylori* in the oral cavity within a Mexican population and its relationship with gastroesophageal disease.

Material and methods

Study population

A total of 131 individuals from two different populations with different characteristics were studied. The first cohort consisted of 66 randomly selected, hospitalized individuals (mean age 45 years, 64% female) from the National Institute of Respiratory Diseases (INER), a specialized hospital belonging to the Mexican Public Health Council (Secretary of Health). The majority of these patients had been diagnosed with a clinical and/or histological gastroesophageal disease as follow: 16 suffering from non-ulcerous dyspepsia (NUD), 30 with gastroesophageal reflux disease (GERD), 10 with gastric cancer (GC), and another 10 with a different non-gastric pathology (ONGP). The second cohort consisted of 65 randomly selected, non-hospitalized, ambulatory individuals (mean age 32 years, 54% female), who attended the dental clinic of the Faculty of Odontology at the National Autonomous University of Mexico (UNAM) for dental cleaning. The second cohort underwent a physical examination and was given a questionnaire by a gastroenterologist; the diagnostic results for this population were as follows: 20 individuals had NUD, while the remaining 45 were apparently healthy patients (AHP). Ethics committee approval and informed consent were obtained from the individuals of both cohorts.

Collection of clinical specimens

Gastric biopsy, saliva and dental plaque samples were obtained from hospitalized individuals (66 patients) who underwent endoscopy for upper gastrointestinal complaints at the Endoscopy Unit of the National Institute of Respiratory Diseases. Both dental plaque and saliva samples were collected from the patients in the morning prior to undergoing endoscopy. Only saliva and dental plaque samples were simultaneously taken from ambulatory individuals (65 patients, UNAM). Gastric biopsy was not obtained from this last population to comply with ethical standards. Dental plaque samples were obtained by scraping the dental surface, supra and infragingival, with sterilized Gracey curettes that were then suspended in collecting tubes containing isotonic saline solution. Saliva samples were collected by the direct dropping of saliva into sterilized collecting tubes. Collecting tubes for both types of samples were immediately put on ice for transportation to the laboratory before processing. The biopsies were transported to the laboratory in Brucella broth with 10% glycerol and frozen at −70°C until tested.

PCR-based *H. pylori* detection

Direct DNA extraction from gastric biopsies was carried out using the boiling method. Briefly, the biopsy specimen was homogenized with a glass tissue grinder and 50 μl of isotonic saline solution was added, the sample was transferred to a micro-centrifuge tube, which was put into multi-block heat at 100°C for 10 min to be used as the genomic target for PCR. DNA extraction from dental plaque and saliva samples were obtained by the same method. The *vacA* gene was chosen to detect *H. pylori* since *vacA* is a specific and constitutive *H. pylori* gene (2). PCR was performed as previously indicated by Atherton (2). Briefly, each sample was typed as vacA signal region, s1 or s2, by performing a PCR assay using the conserved forward and reverse primers VA1-F (5′ ATGGAAATACAACAAACACAC 3′) and VA1-R (5′CTGCTTGAATGCGCCAAAC 3′), respectively. These primers differentiate the amplification products on the basis of size and categorize them into type s1 (259 bp) and type s2 (286 bp). The *H. pylori* 8823 and 8822 strains were used as a positive control for s1 and s2, respectively, and one *E. coli* K12 strain was included as a negative control. Conditions for thermal cycling were 35 cycles at 94°C for 1 min, 52°C for 1 min and 72°C for 1 min. Amplification products obtained were electrophoresed on 1.2% agarose gels and UV visualized.

Statistical methods

Frequency of *H. pylori* detection by PCR in saliva and dental plaque from both cohorts, and its association with gastric detection and clinical presentation of gastroesophageal disease, were compared using the χ^2 test with Yates' continuity correction or Fisher's exact test.

Results

Frequencies of *H. pylori* detection by PCR from gastric biopsy, saliva and dental plaque samples for each cohort can be seen in Table 1. The presence of *H. pylori* in hospitalized patients according to sample type was as follows: the gastric biopsy for 40 (61%) patients was positive; 16 (24%) patients had positive saliva samples;

Table 1. Frequency of *Helicobacter pylori* detection in oral cavity samples from two patient cohorts with different clinic conditions

		PCR detection in		
		Saliva	Dental plaque	Gastric biopsy
Clinical diagnosis	No. subject	No. (%)	No. (%)	No. (%)
National Institute of Respiratory Diseases[1]				
NUD	16	8 (50)	11 (67)	13 (81)
GERD	30	5 (17)	18 (60)	20 (67)
CA	10	3 (30)	5 (50)	6 (60)
ONGP	10	0 (0)	0 (0)	1 (10)
Total	66	16 (24)	34 (52)	40 (61)
National Autonomous University of Mexico[2]				
NUD	20	10 (50)	13 (65)	ND
AHP	45	0 (0)	0 (0)	ND
Total	65	10 (15)	13 (20)	ND

[1]Hospitalized and [2]ambulatory populations.
NUD, non-ulcerous dyspepsia; GERD, gastroesophageal reflux disease; CA, cancer; ONGP, other non-gastric pathology; AHP, apparently healthy patients; ND, not determined.

and 34 (52%) had positive dental plaque samples; with regards to the ambulatory cohort: 10 (15%) had positive saliva samples and 13 (20%) had positive dental plaque samples.

Separate analysis for each cohort showed that in the hospitalized population there was an association between gastroesophageal disease and *H. pylori* detection in gastric biopsies ($P = 0.0006$) and the detection of *H. pylori* in dental plaque ($P = 0.0003$). However, the detection of *H. pylori* in saliva samples showed no statistical significance with gastroesophageal diseases. With respect to sensitivity of each clinic sample for *H. pylori* detection, irrespective of clinical status, there was no statistically significant difference ($P = 0.3807$) between dental plaque samples and gastric biopsies but there was statistical significance when comparing the detection of *H. pylori* from gastric biopsies or dental plaque vs. saliva samples ($P = 0.0001$ and $P = 0.0003$, respectively). With respect to the ambulatory cohort, there was no statistically significant difference between *H. pylori* detection from dental plaque and from saliva samples ($P = 0.6458$).

Separate analysis carried out for each cohort between the detection of *H. pylori* from gastric biopsy, dental plaque and saliva when considering concurrent gastric disease associated with *H. pylori* showed varying outcomes: in the case of the hospitalized cohort, there was a statistical significance in the detection of *H. pylori* from gastric biopsy ($P = 0.0008$), dental plaque (0.0007) and saliva (0.0095) when comparing NUD vs. ONGP; statistically significant differences were found when comparing the detection of *H. pylori* from gastric biopsy ($P = 0.0028$) and dental plaque ($P = 0.0008$) for GERD vs. ONGP. Additionally, a statistically significant association was seen when comparing the detection of *H. pylori* from dental plaque only ($P = 0.0325$) for CA vs. ONGP, and from saliva only for NUD vs. GERD ($P = 0.0362$). No statistical differences were found for gastric biopsy, dental plaque and saliva samples when comparing NUD vs. CA and GERD vs. CA. In the case of the ambulatory cohort, there was a statistically significant association ($P = 0.0001$) between *H. pylori* detection from both saliva and dental plaque samples with those clinically diagnosed with NUD.

Statistical analysis for each population was carried out to see if there was any clear association between *H. pylori* detection from gastric biopsy and/or saliva and/or dental plaque with the sex and clinical status of the individual. For the hospitalized cohort, *H. pylori* detection was highly significant in saliva samples from females who had been diagnosed with NUD compared with that of the male participants ($P = 0.028$). There were no other differences found relating to other gastric disease and sex of the individuals within this population. In the ambulatory cohort, there were no significant differences regarding these characteristics.

With respect to the choice of the primers used for *H. pylori* detection from dental plaque and saliva samples, VA1-F and VA1R were highly specific and sensitive for dental plaque and saliva samples as has been demonstrated for gastric biopsy previously (1, 2, 6, 12, 19, 21). Furthermore, it was possible to characterize the type of *vacA* signal sequence of *H. pylori* strains present in saliva, dental plaque and gastric biopsy samples (Fig. 1). With respect to type of signal sequence detected between both populations studied, approximately 95% of patients from INER and UNAM presented type s1 of the *vacA* gene in their gastric biopsy (INER), dental plaque and/or saliva samples, and only two patients presented PCR products corresponding to type s1 and s2 in all samples tested.

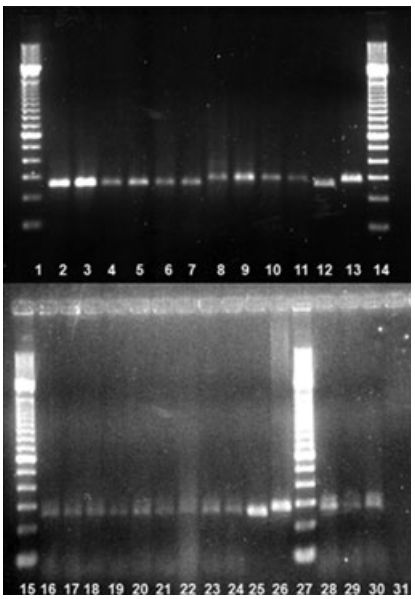

Fig. 1. Gel electrophoresis pattern of *Helicobacter pylori vacA* genotypes amplified by VA1-F and VA1-R primers directly from gastric biopsy (lines 2–11), from dental plaque (lines 16–20, 28) and from saliva (lines 21–24, 29, 30). Molecular marker of 100 bp ladder (lines 1, 14, 15 and 27); positive control type s1 (lines 12 and 25); positive control type s2 (lines 13 and 26); negative control (line 31).

Discussion

There is a clear association between *H. pylori* infection with gastritis, gastric and duodenal ulcer, and the development of gastric cancer (2, 7). However, the association between *H. pylori* detection in the oral cavity and gastric pathology associated with *H. pylori* is still controversial (5, 14, 16, 20, 26, 27). This is in part due to the limited success in culturing *H. pylori* from oral samples in contrast to satisfactory cultures from gastric biopsies (17, 19), combined with the difficultly of establishing if the *H. pylori* strain isolated from the oral cavity is the same *H. pylori* strain as that from the stomach in the same patient (16). Advances in molecular technology have led to the standard use of polymerase chain-reaction (PCR) techniques for the detection of pathogens allowing for a rapid characterization and detection of even small numbers of specific bacteria using specific primers for amplification of a species-specific region of DNA. To date, different primers and PCR protocols have been used to detect *H. pylori* from oral cavity samples, but the results have been very variable (5, 11, 13 15, 23, 26, 27). While some studies showed a low or no *H. pylori* detection in the oral cavity others showed high detection rates (9, 10, 13–15, 17, 18, 20). Similarly, there has been no consistency between the isolation of *H. pylori* from gastric biopsies and the detection of bacteria in the oral cavity in the same study group (5, 14, 16, 26). The inconsistency of these results is probably due to the type of oral sample analyzed (dental plaque and saliva), to the specificity and sensitivity of primers used for *H. pylori* detection by PCR, and to the cohorts studied. Such variability in results has prevented researchers from studying, analyzing and comparing infections of both anatomical sites and much less any association between *H. pylori* detection in the oral cavity and any gastric pathology (5, 14, 16, 26). In the current study, the results clearly show the association of *H. pylori* gastric infection with NUD and GERD. There was no statistical difference in *H. pylori* detection between gastric biopsy and dental plaque samples from patients with any gastroesophageal diseases. In terms of the two oral samples used in the study, *H. pylori* was better detected from dental plaque than from saliva, probably due to the fact that dental plaque provides a solid matrix that allows the bacteria to maintain adherence, while the continuous flow and constant production of saliva

prevents the establishment of a sufficient bacterial load making bacterial detection difficult. It is also important to take into account the specificity and sensitivity of selected primers used to identify the bacteria by PCR, especially from regions like the mouth where there is a complexity of oral microflora. In the current study, a set of VA1-F and VA1-R primers were selected, which are widely used in the genetic characterization of *H. pylori*. As previously described by Atherton (2), these primers amplify the signal sequence (ss) region of the *vacA* gene, a specific and structural gene that encodes *H. pylori* vacuolizing cytotoxin (VacA) (1, 2, 6, 12, 19, 25). The referred primers have been used successfully for genotyping *H. pylori* strains in cultures previously isolated as also directly from the DNA of gastric biopsy sample without needing to isolate the bacteria (6, 21). The specificity and sensitivity of the primers have been widely demonstrated (1, 2, 6, 12, 19, 21). Chattopadhyay et al. in 2004, showed the sensitivity of these primers detecting the different genotypes s1 and s2 when simulated a mixed infection with two *H. pylori* strains with different genotypes, strain 26695 (*vacA* s1m1) and strain I-80 (*vacA* s2m2), these strains were serially diluted at rations from 1 : 1 to 1 : 32. Multiplex PCR performed with the serially diluted culture mixtures detected mixed genotypes, with I-80/26695 ranging from 8 : 1 to 1 : 16 (6). The current study showed the primers specificity for *H. pylori* by PCR with the two reference strains 8822 (type s2) and 8823 (type s1) as positive control, but amplification product was not detected for the *E. coli* k12 strain (negative control). Furthermore, these primers let us to determine the type s1 or s2 of the *vacA* signal region for each amplified product (Fig. 1). It was not considered necessary to use other primers that characterize the *vacA* middle region (3) because it was not the aim of this study, but we are sure that these primers are as sensitive as VA1-F and VA1-R for determining the *H. pylori vacA* genotype completely, in addition, other primers can be used to determine marker genes as *cagA* and *cag*-PAI in the *H. pylori* strains present in oral cavity. Finally, a strong association between *H. pylori* presence in the oral cavity was found with gastroesophageal pathology (NUD, GERD and GC) ($P = 0.0001$), while there was no *H. pylori* detection in the oral cavity of the apparently Healthy subjects and with other non-gastric pathologies.

Some authors support the theory that the oral cavity, and more specifically, the dental plaque is a reservoir for *H. pylori* enabling it to persist for a long time and be responsible for the transmission of the bacteria from the oral to the gastric cavity (22), and possibly serve as a source of re-infection after eradication treatment (8, 10, 18). However, it is few probable since the number of bacteria detected in dental plaque is very small (1–213 *H. pylori* cells/mg of dental plaque) (24) and although the number of *H. pylori* organisms necessary to induce infection and disease in the stomach is still unknown, it is known the concentration of *H. pylori* in gastric mucosal biopsy in the range of 5×10^3 to 5×10^5 bacteria per biopsy sample (21) making a significant difference between bacterial density of both anatomic sites, the *H. pylori* low quantity in oral cavity would be insufficient for infecting stomach. In addition, the pass of *H. pylori* from stomach to oral cavity may cause the transformation of the bacteria to a coccoid shape (viable but non-culturable) (4), which is non-infectious making more difficult that the bacteria will be the responsible of re-infections. On the other hand, the presence of a viable coccoid form in oral cavity, which is unculturable by conventional techniques, may explain the limited success to culture *H. pylori* from oral samples. We believe that oral cavity infection by *H. pylori* is secondary to NUD and GERD as the result of gastric reflux, a symptom common in these type of diseases.

Conclusion

This study shows that there is a strong and clear association between *H. pylori* presence in the oral cavity and gastroesophageal disease ($P = 0.0001$) associated with this microorganism, while healthy subjects and those with other non-gastric disease do not present with *H. pylori* in their oral cavity. There was not difference in *H. pylori* detection between gastric biopsy and dental plaque among the patients with gastroesophageal disease. There is a significant difference in *H. pylori* detection in samples from the oral cavity depending on the type of sample chosen; a dental plaque sample is better than a saliva sample. The primers VA1-F and VA1-R used to detect *H. pylori* in the oral cavity have shown to be as sensitive as when they are used for gastric samples and is therefore recommended for detecting *H. pylori* in oral cavity samples. In addition, they can use for genotyping the oral strains. We suggested that detection of *H. pylori* by PCR in oral cavity would be an indirect diagnostic method of gastroesophageal disease associated to *H. pylori*.

Acknowledgments

We are grateful to Elsa Evaristo-Montes for the technical support. This research was funded in part by DGAPA IN206808 from National Autonomous University of Mexico.

References

1. Atherton JC. Molecular techniques to detect pathogenic strains of *Helicobacter pylori*. In: Clayton CL and Mobley HLT, ed. Methods in molecular medicine. *Helicobacter pylori protocols*. Totowa, NJ: Humana Press Inc., 1997: 133–143.
2. Atherton JC, Cao P, Peek RM Jr, Tummuru MKR, Blaser MJ, Cover TL. Mosaicism in vacuolatin cytotoxin alleles of *Helicobacter pylori.* Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 1995: **270**: 1771–1777.
3. Atherton JC, Cover TL, Twells RJ, Morales ER, Hawkey JC, Blaser MJ. Simple and accurate PCR-based system for typing vacuolating cytotoxin alleles of *Helicobacter pylori*. J Clin Microbiol 1999: **37**: 2979–2982.
4. Azevedo NF, Almeida C, Cerqueira L, Dias S, Keevil CW, Vieira MJ. Cocooid form of *Helicobacter pylori* as a morphological manifestation of cell adaptation to the environmental. Appl Environ Microbiol 2007: **73**: 3423–3427.
5. Butt AK, Khan AA, Izhar M, Alam A, Shah SW, Shafqat F. Correlation of *Helicobacter pylori* in dental plaque and gastric mucosa of dyspeptic patients. J Pak Med Assoc 2002: **52**: 196–200.
6. Chattopadhyay S, Patra R, Ramamurthy T et al. Multiplex PCR assay for rapid detection and genotyping of *Helicobacter pylori* directly from biopsy specimens. J Clin Microbiol 2004: **42**: 2821–2824.
7. Cover TL, Blaser MJ. *Helicobacter pylori*: a bacterial cause of gastritis, peptic ulcer disease and gastric cancer. ASM News 1995: **61**: 21–26.
8. Desai HG, Gill HH, Shankaran K, Metha PR, Prabhu SR. Dental plaque: a permanent reservoir of *Helicobacter pylori*? Scand J Gastroenterol 1991: **26**: 1205–1208.
9. Dowsett SA, Kowolik MJ. Oral *Helicobacter pylori*: can we stomach it? Crit Rev Oral Biol Med 2003: **14**: 226–233.
10. Gebara EC, Fartia CM, Pannuti C, Chehter L, Mayer MP, Lima LA. Persistence of *Helicobacter pylori* in the oral cavity alters systemic eradication. J Clin Periodontol 2006: **33**: 329–333.
11. Goosen C, Theron J, Ntsala M et al. Evaluation of a novel heminested PCR assay based on the phosphoglucosamine mutase gene for detection of *Helicobacter pylori* in saliva and dental plaque. J Clin Microbiol 2002: **40**: 205–209.
12. González-Valencia G, Atherton JC, Muñoz O, Dehesa M, Madrazo de la Garza A,

Torres J. *Helicobacter pylori vacA* and *cagA* genotypes in mexican adults and children. J Infect Dis 2000: **182**: 1450–1454.
13. Kabir S. Detection of *Helicobacter pylori* DNA in feces and saliva by polymerase chain reaction: a review. Helicobacter 2004: **9**: 115–123.
14. Kamat AH, Mehta PR, Natu AA, Phadke AY, Vora IM, Desai PH. Dental plaque: an unlikely reservoir of *Helicobacter pylori*. Indian J Gastroenterol 1998: **17**: 123–125.
15. Li C, Ha T, Ferguson DA 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**: 2142–2149.
16. Loster BW, Majewski SW, Czesnikiewicz-Guzik M, Bielanski W, Pierzchalski P, Konturek SJ. The relationship between the presence of *Helicobacter pylori* in the oral cavity and gastric in the stomach. J Physiol Pharmacol 2006: **57**: 91–100.
17. Luman W, Alkout AM, Blackwell CC, Weir DM, Plamer KR. *Helicobacter pylori* in the mouth-negative isolation from dental plaque and saliva. Eur J Gastroenterol Hepatol 1996: **8**: 1–2.
18. Majmudar P, Shah SM, Dhunjibhoy KR, Desai HG. Isolation of *Helicobacter pylori* from dental plaques in healthy volunteers. Indian J Gastroenterol 1990: **10**: 33–34.
19. Morales-Espinosa R, Castillo RG, González-Valencia G et al. Colonization of Mexican patients by multiple *Helicobacter pylori* strains with different *vacA* and *cagA* genotypes. J Clin Microbiol 1999: **37**: 3001–3004.
20. Oshowo A, Tunio M, Gillam D, Botha AJ, Holton J, Boulos P. Oral colonization is unlikely to play an important role in *Helicobacter pylori* infection. Br J Surg 1998: **85**: 850–852.
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. Pytko-Polonczyk J, Konturek SJ, Karczewska E, Bielanski W, Kaczmarczyk-Stachowska A. Oral cavity as permanent reservoir of *Helicobacter pylori* and potential source of reinfection. J Physiol Pharmacol 1996: **47**: 121–129.
23. Song Q, Haller B, Schmid RM, Adler G, Bode G. *Helicobacter pylori* in dental plaque. A comparison of different PCR primers sets. Dig Dis Sci 1999: **44**: 479–484.
24. Song Q, Haller B, Ulrich D, Wichelhaus A, Adler G, Bode G. Quantitation of Helicobacter ptlori in dental plaque samples by competitive polymerase chain reaction. J Clin Pathol 2000: **53**: 218–222.
25. Salama NR, Gonzalez-Valencia G, Deatherage B et al. Genetic analysis of Helicobacter pylori strain populations colonizing the stomach at different times postinfection. J Bacteriol 2007: **189**: 3834–3845.
26. Teoman I, Ozmeric N, Ozcan G et al. Comparison of different methods to detect *Helicobacter pylori* in the dental plaque of dyspeptic patients. Clin Oral Investig 2007: **11**: 201–205.
27. Tiwari SK, Khan AA, Ahmed KS et al. Rapid diagnosis of *Helicobacter pylori* infection in dyspeptic patients using salivary secretion: a non-invasive approach. Singapore Med J 2005: **46**: 224–228.
28. Torres J, Leal Y, Pérez-Pérez G et al. A community-based seroepidemiologic study of *Helicobacter pylori* infection in Mexico. J Infect Dis 1998: **178**: 1089–1094.