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Virulence genes of *Helicobacter pylori* in Côte d'Ivoire

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Abstract
Objective: To determine prevalence of vacA, cagA and oipA genotypes in gastric biopsies in Abidjan (Côte d'Ivoire).
Material and methods: A total of 98 positive urea rapid-biopsy specimens in an endoscopy room at Hospital and University Center of Cocody (Abidjan) for period from August 2015 to January 2016 were selected for the study. Gastric biopsies were collected and sent to Laboratory of Pasteur Institute of Côte d'Ivoire under conditions and delays of routing. Genes were detected by PCR.
Results: Gastric biopsies that had positive PCR to one or more genes were 71.4% (70/98). CagA and oipA genotypes were detected at 72.8% and 37.2%, respectively. VacA s1a region was identified in 15.7% (11/70) and the VacA m1a region in 5.7% (4/70). Taken together, vacA s1a / m1a allele was identified at 55.7% (39/70). 61.5% of patients were women and 38.5% of men with a sex ratio of 0.62. Average age was 41.7 years (between 19 and 72 years).
Conclusion: High prevalence of infection by virulent factors could be used to discern risk of developing severe gastroduodenal diseases in host and contribute to characteristics of *H. pylori* infection.

Keywords: *Helicobacter pylori*, virulence genes, Côte d'Ivoire

1. Introduction

Helicobacter pylori (H. pylori) colonizes stomach of more than 50% of human population in world and is probably one of most widespread bacterial pathogens [1-3]. Presence of *H. pylori* in the gastric mucosa is now associated with development of pathologies such as gastric or duodenal ulcer, chronic gastritis, MALT lymphoma or gastric cancer [4]. Research to elucidate the determinants of disease progression has revealed genetic diversity in the bacterium that increases risk of developing gastric cancer. Main virulence factors of H. pylori are related to cytotoxin-associated gene (cagA) [5, 6], vacuolating associated cytotoxin (vacA) gene [7, 8] and outer membrane protein gene (oipA) [9, 10]. *H. pylori* strains are often classified as cagA + or cagA- strains, depending on whether or not cagA bacterial protein encoded by cagA gene [11]. This protein of 120 to 240 kDa is injected into epithelial cell by Type IV secretion system (TIVSS). Once released into host cell, cagA protein is phosphorylated by Abl and Src family tyrosine kinases of glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) motifs which, after interaction with phosphatase SHP- 2 causes dephosphorylation of cortactin and rearrangement of skeleton at gastric mucosa. This will lead to cellular morphological alterations called "hummingbird beaks". Several studies have shown a close link between this cellular motility and oncogenic transformation [12, 13]. VacA protein is encoded by vacA gene present in all strains of H. pylori. This highly immunogenic protein induces in vitro intracellular vacuolation [14] and is capable of immunosuppression by blocking activation of T lymphocytes which contributes to persistence and longevity of infection [15, 16]. VacA sequence shows a polymorphism in two regions, central region (m1 and m2 alleles) and region coding for signal sequence (alleles s1 and s2). Strains associating s1/m1 alleles show a strong cytotoxic activity whereas those of genotype s2/m2 which have no cytotoxic activity [17, 18]. Presence of functional oipA gene in *H. pylori* is strongly associated with duodenal ulcers, gastric cancer and increased infiltrations of neutrophils [19, 20]. OipA belongs to outer membrane protein family and is associated with high production of interleukin 8 (IL-8) *in vitro* [21]. It also plays a role in induction of gastric inflammation and production of inflammatory cytokines IL-1, IL-17 and tumor necrosis factor alpha (TNF-α) in gerbils of Mongolia [22]. It contributes to induction of matrix metalloproteinase 1 (MMP-1) strongly associated with gastric cancer [23]. This gene is located at approximately 100kb of pathogenicity island Cag on *H. pylori* chromosome [24]. In Côte d'Ivoire, data on these *H. pylori* genotypes are rare and there are no data to date on vacA and oipA genes. Our study is the first of its kind to simultaneously study several genotypes and aims to determine biodiversity of *H. pylori* virulence genotypes from gastric biopsies.

2. Material and methods

Gastric biopsies

A total of 98 positive urea urea-positive biopsies in endoscopy room at Hospital and University Hospital center of Cocody for period from August 2015 to February 2016 were selected for study. Samples were sent to Bacteriology-Virology laboratory of Pasteur Institute of Côte d'Ivoire under conditions and within a maximum of 4 hours. Biopsies were then stored in dry tubes at -80 °C.

Ethics
All patients asked a socio-demographic questionnaire (age, sex, occupation) and medical history validated by Ethics Committee of Pasteur Institute of Côte d'Ivoire. Written consent was also given by each patient before endoscopy.

Extraction of *H. pylori* DNA
Extraction of *H. pylori* DNA was performed according to DNA extraction protocol of NucliSENS® kit with some modifications. Biopsies were ground in 0.3 ml of 1X PBS buffer with Potter grinder into a sterile tube and then suspended in 500µl of buffer containing Tris-HCl 10 mM, EDTA 1 mM pH 8.0, Proteinase K 1 mg /ml and incubated at 60 ° C for 24 h. DNA was extracted in 500 µl of lysis buffer containing 20 mM Tris, 2 mM EDTA, 150 mM NaCl, 1% SDS and Proteinase K 100 µg/ml for 1 h at 60°C. 1 ml of phenol-chloroform-iso-amyl alcohol mixture (25:24:1) was added and centrifuged at 13000 rpm for 15 min. Aqueous phase (upper phase) was collected and 1/ 10^{th} of 3M sodium acetate and 500µl of absolute ethanol were added and incubated 1 at -80°C for 1 h or overnight at -20°C. The pellet obtained is washed with 70% ethanol and dried at 65°C for 15 min. Pellet obtained is eluted in 60 µl of buffer and DNA is stored at -20°C.

***H. pylori* genotyping**
PCR was carried out in a volume of 50 µl containing 0.75 µl of each 10 mM primer, 3 µl of genomic DNA, 1 µl of 10 mM dNTPs, 3 µl of 25 mM $MgCl_2$, 5 µl of each colored and colorless buffer 0.3 µl of Taq polymerase (Promega (R)). Amplification was carried out in an automaton thermocycler (Biometra® UNO II). Table 1 summarizes sequences of primers used and different sizes expected. After initial denaturation of 94 ° C. for 5 min, amplification for cagA and vacA genes was 35 cycles of 94 ° C: 1 min; 52 ° C: 1mim; 72 ° C: 1min. Each cycle had a final elongation phase of 72 ° C. for 7 min. For oipA gene, after an initial denaturation step of 94 ° C for 5 min, cycle was also 35 cycles of 94 ° C: 1min; 56 ° C: 1min; 72 ° C: 1 min with a final elongation step of 72 ° C for 7 min. Migration of PCR products was performed on 1.5% agarose gel and detection by a GelDoc ™ XR System (Bio-Rad Laboratories, Hertfordshire, UK). A negative control which did not contain DNA was used for quality control of amplifications.

Table 1: PCR primers for amplification of vacA, cagA and oipA sequences

Region	Primers	Sequences	sizes (bases pairs (bp))	References
CagA	D008 R008	5'ATAATGCTAAATTAGACAACTTGAGCG-3' 5'-TTAGAATAATCAACAAACATCACGCCA-3'	298	[25]
VacA S	SIGF SIGR	5'-ATGGAAATACAACAAACACACCG-3' 5'-CAACCTCCATCAATCTTACTGGA-3	338	[26]
M	VA6-F VA5-5	5'-TCAATATCAACAAGCTC-3' 5'-CCGCATGCTTTAATGTC-3'	787	[27]
s1a	SS1-Fa VA1-R	5'-GTCAGCATCACACCGCAAC-3' 5'-CTGCTTGAATGCGCCAAAC-3'	190	[27]
m1a	VA3-F VA3-1R	5'- GGGTCAAAATGCGGTCATGG-3' 5'-CTGTTAGTGCCCGCAGAAAC-3	290	[27]
OipA	HP0638F HP0638R	5'-GTTTTTGATGCATGGGATTT-3' 5'-GTGCATCTCTTATGGCTTT-3'	401	[28]

Statistical method
Data were entered and described using software called Epi-info version 3.5.4. These data were then transcribed into an Excel database to facilitate a single and varied analysis. Statistical tests were interpreted at significance level corresponding to an alpha risk of 5%. Qualitative variables were compared using Pearson Chi-2 test or Fisher's exact test when one of variables was less than 5.

3. Results
Prevalence of detected genes and general characteristics of patients with genotypes
Biopsies that had positive PCR to one or more genes were 71.4% (70/98) and 28.6% (28/98) were negative for all genes. The population of patients with genotypes was predominantly female with 61.4% (43/70). Men accounted for 38.6% or 27/70. Average age was 41.7 years with a minimum of 19 years and a maximum of 72 years. The most represented age group was that of patients aged between 31 and 40 years. Although some genotypes were detected more in one sex than in other, their presence was not significantly associated with age or sex of patients ($p > 0.05$).

VacA genotype
Sizes of amplification products for vacA s1a and vacA m1a were 190 bp and 290 bp respectively. VacA s1a region was identified in 15.7% (11/70) and the vacA m1a region in 5.7% (4/70). Together, the s1a / m1a allele was identified at 55.7% (39/70). A proportion of 22.9% (16/70) could not be sought in our study.

CagA genotype
CagA genotype was detected at 72.8% (51/70). Gene was absent in 19/70 or 27.8%. 298 bp amplification products indicating the presence of the cagA are shown in FIG. 1A.

Genotype of oipA
OipA genotype was detected at 37.2% (26/70). Amplification products of 401 bp indicating presence of oipA are shown in FIG. 1B.

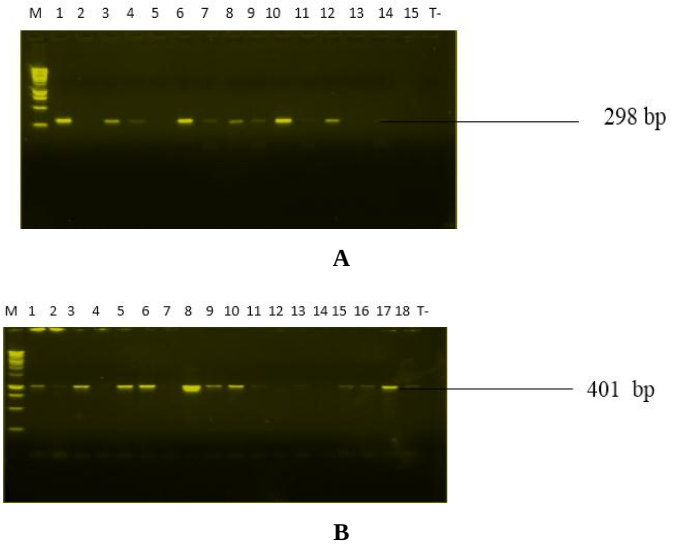

Fig 1: Genotyping of cagA and oipA by PCR. **A:** CagA genotype. M: size marker, 250 bp. Line 1, 3, 4, 6-12: cagA positive gene. Line 2, 5, 13-15: cagA negative gene. **B:** Genotype of oipA. M: size marker, 100 bp. Line 1-3,5,6, 8-11,13, 15-18: oipA positive gene. Line 3,7,12 and 14: oipA negative gene. T-: negative control without DNA.

Distribution of genotypes according to endoscopic aspect

All genotypes were mainly identified in cases of erythematous gastropathy and pangastropathy (Table 2).

Table 2: Frequency of identified genotypes according to endoscopic aspect

Endoscopic aspect	Genotypes		
	S1a+/m1a+	cagA+	oipA+
Gastropathy with gastric reflux	8(20,6%)	6(11,8%)	3(11,5%)
erythematous gastropathy	20(51,3%)	29(56,8%)	15(57,7%)
Pangastropathy	9(23,1%)	13(25,5%)	7(26,9%)
Other[(1)]	1(2,6%)	2(3,9%	1(3,9%)
Normal	1(2,6%)	1(2%)	0(0%)
Total	39(100%)	51(100%)	26(100%)

[(1)]Other: Savary Miller's stage 2 esophagitis, congestive duodenopathy

Genotypes and family history of ulcer syndrome

41/70 (58.6%) of patients with genes had a family history of ulcer syndrome. Presence of VacA s1a+/m1a+ genotypes was significantly associated with patients with a family history of ulcer syndrome (p = 0.012).

Combined genotypes according to endoscopic aspect

Based on search for cagA gene, vacA region (s1a and m1a) and oipA gene, five different genotypic combinations were identified. The most common genotype was s1a+/m1a+/cagA+/oipA- at 30% (21/70) and majority of combined genotypes were observed in erythematous gastropathy and pangastropathy (Table 2).

Table 2: Frequency of the different genotypic combinations observed according to the endoscopic aspect

Endoscopic aspect	Combined genotypes				
	S1a+/m1a+/ cagA+/oipA+	**S1a+/m1a+/ cagA+/oipA-**	**S1a+/m1a+/ cagA-/oipA+**	**S1a+/m1a-/ cagA+/oipA+**	**S1a-/m1a+/ cagA+/oipA+**
Gastropathy with gastric reflux	0(0%)	5(23,8%)	1(25%)	0(0%)	0(0%)
Erythematous gastropathy	8 (88,9%)	7(33,3%)	2(50%)	1(100%)	0(0%)
Pangastropathy	1(11,1%)	7(33,3%)	1(25%)	0(0%)	1(100%)
Other[(1)]	0(0%)	1(4,8%)	0(0%)	0(0%)	0(0%)
Normal	0(0%)	1(4,8%)	0(0%)	0(0%)	0(0%)
Total	9(100%)	21(100%)	4(100%)	1(100%)	1(100%)

[(1)] Other: Savary Miller's stage 2 esophagitis, congestive duodenopathy.

4. Discussion

The present study reports that genotypes vacA, cagA and oipA have been identified in gastric biopsies. A female predominance with a sex ratio of 0.62 male/female was reported. Similar results have been reported by Ben Mansour *et al.* [29] in Tunisia. However, no study in our knowledge has demonstrated a link between sex and presence of virulence genes. Majority of genotypes were identified in patients aged between 31 and 40 years. This average age is by far less than that of developed countries which is around 60 years [30]. This confirms that in developing countries *H. pylori* contamination is early. Indeed, several studies tend to show that an individual has very little risk of being infected after the age of 10 years [31]. Risk factors for acquiring *H. pylori* infection are frequently associated with poverty. They include promiscuity, lack of hygiene, bed sharing in childhood, and low parental education [32].

Predominant genotype in gastric biopsies was cagA + genotype (72.8%). Our results were in agreement with those of Senegal, which reported a prevalence of 73.3% of cagA genotype [33], while Ben Mansour *et al* reported a prevalence of 61.6% of cagA genotype [29]. This shows geographical influence on adaptation of organism to its environment and climatic conditions. Indeed, concerning Côte d'Ivoire and Senegal there is a geographical and climatic proximity between these two countries in relation to Tunisia. For vacA genotype, strongly associated vacA s1a+/m1a+ allele was high (55.7%). A study in South Africa reported absence of this allele in isolated *H. pylori* strains [34]. This explains well disparity in geographical distribution of *H. pylori* strains [35].

In this study, oipA genotype was found at 37.2% while Ben Mansour *et al.* found oipA at 90.8% in Tunisia [29]. This contrast explains adaptability of *H. pylori* to its environment and climatic conditions, but to its host because oipA is strongly involved in immune response in host [36, 37].

All identified genotypes were predominant in cases of erythematous gastropathy and pangastropathy associated with inflammation and lesions in the gastric mucosa that typically characterize *H. pylori* infection [38]. This expresses clearly relationship between gene status and clinical symptoms in populations of patients studied.

Among patients with genotypes, 41/70 (58.6%) had a family history of ulcer syndrome and vacA s1a+/m1a+ genotype was significantly associated (p = 0.012). In other words, same

genotype is found in members of same family because vacA s1a+/m1a+ genotype is a specific subtype of *H. pylori*. These results show clearly notion of human-to-human contamination and that family environment would contribute to spread of bacteria [39].

Combination of different genotypes vacA, cagA and oipA illustrates mosaic composition of *H. pylori* genome. S1a+/m1a+/cagA+/oipA+ genotype was exclusively observed in cases of erythematous gastropathy and pangastropathy. This shows multifunctional character in the mechanism of gastric mucosa infestation using several virulence factors [40]. Also, detection of multiple genotypes involves presence of multiple strains in clinical specimens. Indeed, 22.9% of strains could not be investigated with primers s1a and m1a used in the study. This suggests presence of alleles other than those identified in this study. This would imply that it is possible for more than one strain to be acquired in childhood, especially in countries where prevalence of *H. pylori* is high, however, there is no evidence that several strains colonize simultaneously (co-infection). In addition, several studies have demonstrated that coinfection or superinfection are common [41, 42].

5. Conclusion

VacA, cagA and oipA genotypes of *H. pylori* were identified from gastric biopsies in Abidjan. In Côte d'Ivoire it is possible that the high prevalence of infection with virulent factors contributes to characteristics of *H. pylori* infection and is used to discern risk of development of infection to forms more severe diseases such as gastric cancer.

6. References

1. Blaser MJ. Ecology of Helicobacter pylori in the human stomach. J Clin Invest. 1997; 100:759‑62.
2. Blaser MJ. Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. J Infect Dis. 1990; 161:626‑33.
3. Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori. Clin Microbiol Rev. 1997; 10:720‑41.
4. Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. J Clin Invest. 2004; 11:321-333.
5. Mattar R, Marques SB, Monteiro Mdo S, Dos SA, Iriya K, Carrilho FJ. Helicobacter pylori cag pathogenicity island genes: clinical relevance for peptic ulcer disease development in Brazil. J Med Microbiol. 2007; 56:9-14.
6. Tomasini ML, Zanussi S, Sozzi M, Tedeschi R, Basaglia G, De Paoli P. Heterogeneity of caggenotypes in Helicobacter pylori isolates from human biopsy specimens. J Clin Microbiol. 2003; 41:976-980.
7. Atherton JC. The clinical relevance of strain types of Helicobacter pylori. Gut. 1997; 40:701-704.
8. Cover TL. The vacuolating cytotoxin of Helicobacter pylori. Mol Microbiol 1996, 20:241-246.
9. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of Helicobacter pylori. Proc Natl Acad Sci USA. 2000; 97:7533-7538.
10. Dossumbekova A, Prinz C, Mages J, *et al.* Helicobacter pylori HopH (OipA) and bacterial pathogenicity: genetic and functional genomics analysis of hopH gene polymorphisms. J Infect Dis. 2006; 194:1346-1355.
11. Covacci A, Censini, *et al.* Molecular characterization of the 128-kDaimmunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci USA. 1993; 90(12):5791-5795.
12. Odenbreit S, Puls J, *et al.* Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. Science. 2000; 287(5457):1497-1500.
13. Stein M, Bagnoli F, *et al.* c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol. 2002; 43(4):971-980.
14. Hotchin NA, Cover TL, *et al.* Cell vacuolation induced by the VacA cytotoxin of Helicobacter pylori is regulated by the Rac1 GTPase. J Biol Chem. 2000; 275(19):14009-14012.
15. Gebert B, Fischer W, *et al.* Helicobacter pylori vacuolating cytotoxin inhibits T lymphocyte activation. Science. 2003; 301(5636):1099-1102.
16. Sundrud MS, Torres VJ, *et al.* Inhibition of primary human T cell proliferation by Helicobacter pylori vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proc Natl Acad Sci USA. 2004; 101(20):7727-7732.
17. Miehlke S, Kirsch C, *et al.* The Helicobacter pylori vacA s1, m1 genotype and cagA is associated with gastric carcinoma in Germany. Int J Cancer. 2000; 87(3):322-327.
18. Rhead JL, Letley DP, *et al.* A new Helicobacter pylori vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology. 2007; 133(3):926-936.
19. Yamaoka YO. Ojo, *et al.* Helicobacter pylori outer membrane proteins and gastroduodenal disease. Gut. 2006; 55(6):775-781.
20. Franco AT, Johnston E, *et al.* Regulation of gastric carcinogenesis by Helicobacter pylori virulence factors. Cancer Res. 2008; 68(2):379-387.
21. Yamaoka Y, Kudo T, *et al.* Role of interferon-stimulated responsive element-like element in interleukin-8 promoter in Helicobacter pylori infection, Gastroenterology. 2004; 126(4):1030-1043.
22. Sugimoto M, Ohno T, *et al.* Gastric mucosal interleukin-17 and -18 mRNA expression in Helicobacter pylori-induced Mongolian gerbils. Cancer Sci. 2009; 100(11):2152-2159.
23. Wu JY, Lu H, *et al.* Balance between polyoma enhancing activator 3 and activator protein 1 regulates Helicobacter pylori-stimulated matrix metalloproteinase 1 expression." Cancer Res. 2006; 66(10):5111-5120.
24. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of Helicobacter pylori. Proc Natl Acad Sci USA, 2000; 97:7533-7538.
25. Matsunari O, Shiota S, Suzuki R, Watada M, Kinjo N, Murakami K, *et al.* Association between Helicobacter pylori virulence factors and gastroduodenal diseases in Okinawa, Japan. J Clin Microbiol. 2012; 50:876-83.
26. Van Doorn LJ, Figueiredo C, Sanna R. Expanding allelic diversity of H. pylori vacA. J Clin Microbiol. 1998; 36:2597-2603.
27. Atherton JCCP, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism invacuolating cytotoxin alleles of

Helicobacter pylori. Association of specifc vacA types with cytotoxin production and peptic ulceration. J Biol Chem. 1995; 270:17771-7.
28. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in Eubteria and application to finger printing of bacterial genomes. Nucleic Acids Res. 1991; 19:6823-6831.
29. Ben Mansour K, Fendri C, Zribi M, Masmoudi A, Labbene M, Fillali A, *et al.* Prevalence of Helicobacter pylori vacA, cagA, iceA and oipA genotypes in Tunisian patients. Annals of Clinical Microbiology and Antimicrobials. 2010; 9:10-17.
30. Pilotto A, Malfertheiner P. Review article: an approach to Helicobacter pylori infection in the elderly. Aliment Pharmacol Ther. 2002; 16(4):683-691.
31. Megraud F. Helicobacter pylori en Europe et en Afrique. Différence dans le mode de contamination *et al.* résistance aux antibiotiques. Acta Endoscopica 1998, 28:3.
32. Traoré HA, Maiga MY, Diarra M, Picard E, Dembélé M, Diallo AN, *et al.* Etude anatomo-clinique des gastritis chroniques au Mali. Dakar medical. 1994; 39:207-11
33. Breurec S, Michel R, Seck A, Briss S, Côme D, Dieye FB, *et al.* Clinical relevance of cagA and vacA gene polymorphisms in Helicobacter pylori isolates from Senegalese patients. Clin Microbiol Infect. 2012; 18:153-159.
34. Letley DP, Lastovica A, Louw JA. Allelic diversity of the Helicobacter pylori vacuolating cytotoxin gene in South Africa: rarity of the vacA s1a genotype and natural occurrence of an s2/m1 allele. J ClinMicrobiol. 1999; 37:1203-1205.
35. Vincent P, Gottrand F, Leclerc H. Epidémiologie d'*Helicobacter pylori*: disparités dans la distribution de l'infection. Gastroenterol. Clin. Biol. 1996; 20:S27-33.
36. Lu H, Wu JY, Kudo T, Ohno T, Graham DY, Yamaoka Y. Regulation of interleukin-6 promoteractivation in gastric epithelial cells infected with Helicobacter pylori. Mol Biol Cell. 2005; 16:4954-4966.
37. Yamaoka Y, Kikuchi S, el Zimaity HM, Gutierrez O, Osato MS, Graham DY. Importance of Helicobacter pylori oipA in clinical presentation, gastric inflammation, and mucosal interleukin 8production. Gastroenterology. 2002; 123:414-44.
38. Sibony M, Jones NL. Recent advances in Helicobacter pylori pathogenesis. Cur Opin Gastroenterol. 2012; 28:30-5.
39. Megraud F. *Helicobacter pylori* en Europe et en Afrique. Différence dans le mode de contamination et la résistance aux antibiotiques. Acta Endoscopica. 1998; 28-n°3.
40. George JT, Boughan PK, *et al.* Host anti-microbial response to Helicobacter pylori infection. Mol Immunol. 2003; 40(7):451-456.
41. Van Doorn LJ, Figueiredo C, Rossau R, Jannes G, van Asbroek M, Sousa JC, *et al.* Typing of Helicobacter pylori vacA gene and detection of cagA gene by PCR and reverse hybridization. J Clin Microbiol. 1998; 36:1271-1276.
42. Gonzalez-Valencia G, Atherton JC, Munoz O, Dehesa M, la Garza AM, Torres J. Helicobacter pylori vacA and cagA genotypes in Mexican adults and children. J Infect Dis. 2000; 182:1450-1454.