

Relationship Between Drug Resistance and *cagA* Gene in *Helicobacter pylori*

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Background: Currently, there are evidences for the existence of diverse strains of *Helicobacter pylori* with different levels of virulence. In other way, the most important reason of therapeutic failure is resistance to the antibiotics.

Objectives: The aim of this study was to evaluate the relationship between *H. pylori* drug resistance and the presence of *cagA* gene.

Patients and Methods: Gastric biopsy specimens were cultured for *H. pylori* isolation. Resistance to antimicrobial agents, including clarithromycin, metronidazole, and amoxicillin were detected by modified disk agar diffusion tests. The *cagA* gene was detected using PCR, and the results were analyzed by χ^2 and Fishers exact tests using SPSS software version 16.

Results: A total of 123 *H. pylori*-positive patients (68 males, 55 females, mean age 35 ± 18 years old) were studied. The rate of resistance to clarithromycin, metronidazole and amoxicillin were 17.07, 78.86 and 27.64%, respectively. In this study, the *cagA* was detected in 84 of 123 strains (68.3%). The frequency of resistance to clarithromycin, metronidazole and amoxicillin in *cagA* positive confirmed microorganisms were, 16 (13.01%), 67 (54.47%) and 24 (19.51%), respectively. While the results for *cagA* negative ones were 5 (4.06%), 30 (24.39%) and 10 (8.13%), respectively.

Conclusions: Our results indicated that the overall antibiotic resistance of *H. pylori* was high, and the relationship between drug resistance and *cagA* status was not statistically significant. Therefore, different treatment regimens are not recommended for patients with *H. pylori*-*cagA* positive infections.

Keywords: Antimicrobial Resistance; *cagA* Gene; *Helicobacter pylori*

1. Background

Helicobacter pylori (*H. pylori*) is a microaerophilic spiral-shaped Gram-negative bacterium, which is found in the gastric mucous layer or adherent to the epithelial lining of the stomach (1). The prevalence of *H. pylori* infection is reported as approximately 50%, worldwide, which is as high as 80%-90% in developing countries (2). Although most infected persons remain asymptomatic, but 15-20% of *H. pylori*-positive individuals will develop the associated diseases. The occurrence of diverse diseases as peptic ulcer, gastric cancer and MALT lymphoma with *H. pylori* depends on specific features of the organism, host genetic and environmental factors (3).

H. pylori express the virulence factors that augment the risk of clinical disease outcomes. The virulence factor that has attracted most of the interests is the cytotoxin-

associated gene A (*cagA*), which encodes the CagA protein and the cellular effects of CagA may clarify why patients infected with *cagA* strains, usually have higher inflammatory responses and level of proinflammatory cytokines. Thus, these gastric mucosal responses induce the peptic ulcer diseases, precancerous lesions, and cancer in adults (4). Approximately 60% of *H. pylori* strains isolated in Western countries carry *cag* pathogenesis island (*cag* PAI), whereas almost all of the isolates from East Asia are *cag* PAI-positive (5).

The Maastricht III consensus advised that proton pump inhibitors (PPI), plus clarithromycin, amoxicillin or metronidazole therapy for 7 to 14 days as the first choice for *H. pylori* infection management (6). Recently, several large clinical trials and meta-analyses have shown that the eradication of the standard triple therapy has gener-

Implication for health policy/practice/research/medical education:

The association between peptic ulcer diseases and several virulence genes of *H. pylori* has been described, previously. The aim of this study was to address the relation between the level of *H. pylori* resistance, and the status of the associated virulence associated of *cagA* gene. This kind of study is the first time reported from Iran.

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ally declined to unacceptable levels (i.e., 80% or less) (7). Resistance to antibiotics is the chief factor for treatment failure (2). In other way, results of some studies have suggested that the eradication rate in patients with gastritis is lower than that the patients with peptic ulcer diseases (8). Since the worldwide increase of the drug resistance rates represents a problem of relevance, some researches have been conducted on antibiotic resistance relationship with bacterial genetic factors (9, 10). If harboring *cagA* affects the response rate of therapy, then it may be possible to anticipate *H. pylori* eradication rates.

2. Objectives

The aim of this research was to analyze the relationship between the antibiotic resistance and *cagA* presence in *H. pylori* isolates

3. Patients and Methods

3.1. Patients

One hundred and twenty three *H. pylori* isolates were recovered from gastric biopsy samples of patients with gastritis, peptic ulcer and gastroesophageal reflux diseases underwent endoscopy in Azerbaijan, Iran. At least 1 week prior to endoscopy the patients had stopped receiving antimicrobial agent.

3.2. Bacterial Isolates

For bacterial culture, the gastric biopsy samples were homogenized and cultured onto the Brucella agar (Pro-nadisa Co, Spain) containing 5% sheep blood and antibiotics supplement (vancomycin 6 µg/mL, amphotricin B 2.5 µg/mL and trimethoprim 20 µg/mL). The cultured plates were incubated under microaerophilic condition (Mart, Anoxomat, Lichtenvoorde, Netherlands O₂ 5%, CO₂

10%) at 37°C with high humidity for 5 - 7 days. *H. pylori* was detected based on colony morphology, by Gram staining and positive oxidase, catalase and urease tests.

3.3. Antibiotic Susceptibility Tests

Antimicrobial susceptibility of *H. pylori* isolates were determined by modified disk agar diffusion test. Suspensions of 3-day old cultures were prepared in sterile normal saline to match with No. 4 McFarland standard (11). The disks of antibiotics (MAST Co., England) including clarithromycin (15 µg), amoxicillin (25 µg), and metronidazole (5 µg) were placed on the inoculated plates and incubated for 72 hours. The inhibition zones were interpreted as previously reported (12).

3.4. DNA Extraction

Genomic DNA was extracted with the CTAB reagent method (13). The extracted DNA eluted in 50 µL of 1 × TE buffer (10 mM Tris-HCl, 1 mM EDTA (pH 8.0)) and stored at -20°C until use.

3.5. Assessment of *cagA* and *glmM* Genes by Multiplex PCR

Primer sequences (Table 1) and conditions of PCR amplifications of the *glmM* gene for the detection and confirmation of *H. pylori* and the *cagA* virulent gene were designed based on previous studies (14, 15). Briefly, each PCR was performed in a total volume of 50 µL containing 100 ng *H. pylori* genomic DNA, 0.2 µL of dNTP (10mM), 0.2 µL PCR buffer (1X), 0.1 µL mgcl₂ (1.5 mM), 0.2 µL of each primer and 1.5 µL of Taq polymerase (500 U). The PCR was carried out in an automated thermal cycler (Eppendorf, Germany). After amplification, 10 µL of PCR product was electrophoresed onto 1.5% agarose gel stained with ethidium bromide and later visualized under UV illuminator.

Table 1. The Primer Sequences Used in the Study

Gene	Primer	Nucleotide Sequence	Size (bp)
ureC (glmM)	HP-F	GGATAAGCTTTTAGGGGTGTTAGGGG	294
	HP-R	GCTTACTTTCTAACACTAACGCGC	
cagA	cagA-Fm	AGGGATAACAGGCAAGCTTTTGA	352
	cagA-Rm	CTGCAAAAGATTGTTTGCGAGA	

3.6. Statistical Analysis

The correlations between the *H. pylori-cagA* genotype and antibiotic susceptibilities were analyzed using χ^2 test or Fishers exact test. A P value less than 0.05 was considered as statistically significant.

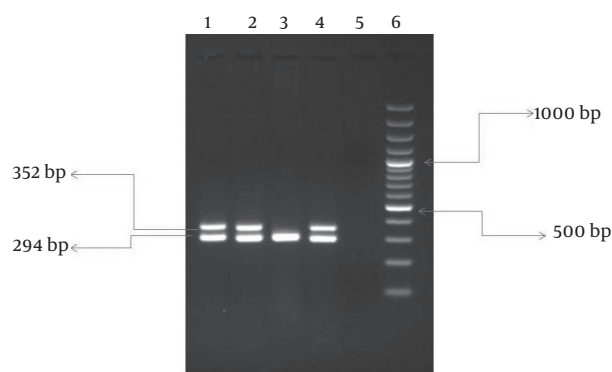
4. Results

The mean age of the patients was 35 ± 18 years. Out of 123

H. pylori isolates, 99 isolates were collected from adults, and 24 isolates from children. Overall, the rate of resistance to clarithromycin, metronidazole and amoxicillin were 17.07, 78.86 and 27.64%, respectively. Resistance to both metronidazole and clarithromycin were detected in 13% (n = 16). Of interest, 10 isolates (8.13%) were resistant to all antibiotics (clarithromycin, metronidazole and amoxicillin). The *cagA* gene was detected in 84 strains (68.3%) (Figure 1). The distribution of the *cagA* gene was the same

in both genders and age groups (73% children and 68% adults, 68.6% male and 68.8% female).

Figure 1. PCR Products of *glmM* and *cagA* Genes by Multiplex PCR



Lanes 1 and 2: patients 2 and 3 that were *glmM* and *cagA* positive, Lane 3: a patient sample with *cagA* negative and *glmM* positive, Lane 4: positive control, Lane 5: negative control, Lane 6: 100 bp DNA ladder.

The presence of *cagA* observed more likely in patients with peptic ulcer diseases (80%) than in non-ulcer dys-

pepsia ones (65%), and the difference was statistically significant (P value ≤ 0.05). The frequency of resistance to clarithromycin, metronidazole and amoxicillin in *H. pylori* - *cagA* positive were, 16 (13.01%), 67 (54.47%) and 24 (19.51%), respectively. While the frequency of resistance to these antibiotics in *H. pylori* - *cagA* negative were 5 (4.06%), 30 (24.39%) and 10 (8.13%), respectively. The distribution of *cagA* in different groups of gastroduodenal diseases and antibiotic resistance are demonstrated in Table 2.

5. Discussion

The three main antibiotics that are used as first-line eradication regimens against *H. pylori* are clarithromycin, metronidazole and amoxicillin (16, 17). However, *H. pylori* resistance to antimicrobials agents is the main cause of treatment failure (18). The level of resistance varies between different geographical regions and unfortunately, which is increasing worldwide. This study showed that the rate of resistance to metronidazole is high (78.86%). In developed countries, metronidazole resistance ranges from 10 to 50% while in developing counties, higher rate of resistance are reported (19). This is may be related to the greater consumption of this drug (2). In the case of

Table 2. Distribution of *cagA* in 123 *H. pylori* Strains Based on Diseases and Antibiotics Susceptibility Patterns

	<i>cagA</i> - Positive, No. (%)	<i>cagA</i> - Negative, No. (%)	Total, No.
Peptic ulcer diseases	16 (80)	4 (20)	20
Non-ulcer diseases	54 (65)	29 (34.9)	83
Am Resistance^a	24 (19.51)	10 (8.13)	34
Cl Resistance^a	16 (13.01)	5 (4.06)	21
Met Resistance^a	67 (54.47)	30 (24.39)	97

^a Abbreviations: Am, amoxicillin; Cl, clarithromycin; Met, metronidazole

clarithromycin, the second most commonly used antibiotic in *H. pylori* management, we found resistance rate as 17.07%. However, in Asian countries, a higher clarithromycin resistance rate has been reported in Japan (40.7%) while, the lowest value has been observed in Malaysia (2.1%) (18). In North America, clarithromycin resistance ranges from 2.5 - 12.2% (20).

In this research, the amoxicillin resistance was found in 27.64% of isolates. Until now, the resistance to amoxicillin in *H. pylori* was reported to be very rare; but have now been reported in USA (21), Italy (22), Brazil (23) and Iran (2, 24). Multiple drug resistance is a major problem worldwide. In the present study, we remarkably observed double resistance to clarithromycin and metronidazole and triple resistance to clarithromycin, metronidazole and amoxicillin. Pretreatment *H. pylori* antibiotic resistance has been reported to compromise the efficacy of management. For example, therapy regimens containing metronidazole and clarithromycin fail in as many as 38%

and 55% of cases, respectively, when used to treat infection with an organism non-susceptible to one of these antimicrobial agents (17).

There are new facts about the existence of various strains of *H. pylori* with different levels of virulence (1, 3, 8, 25-27). The *cagA* gene is located at one end of a 40-kilobase DNA section called *cag* pathogenicity island (*cag* PAI) (28, 29). The clinical relevance of the putative virulence-associated genes of *H. pylori* and geographical region is still a subject of controversy (28). The prevalence of *cagA*-positive *H. pylori* strains varies from one geographic region to another (29). In this research such as the study from Isfahan- Iran (30), the prevalence of *cagA*-positive *H. pylori* strains was 68.3% which is more similar to the findings reported from Western countries (18, 27) and Brazil (31) than those reported from East or Southeast Asia (14, 28, 32). The presence of the *cagA* gene is thought to be associated with a further severe clinical outcome of the disease (3, 27, 33-35). The current study demonstrated the

high prevalence of *H. pylori* infection containing *cagA* in patients from Azerbaijan with peptic ulcer disease than non-ulcer dyspepsia (Pv 0.02). Reports from Turkey (3) and Iraq (36) have shown similar results to our findings. Due to the allelic variation in *cagA* the *H. pylori* subtypes can circulate in different regions, the differences in *cagA* subtype might be a marker for different virulence among *cagA* positive *H. pylori* strains (32).

Furthermore, the severity of gastric inflammation and the pathology also affect the outcome of therapy (8, 9). There is now increasing evidences that *cagA*-positive strains induce an enhanced inflammatory response, mucosal damage and significantly delays healing of ulcers (1, 8). In this study, the rate of resistance to antibiotics in *H. pylori*- *cagA* positive were higher than *cagA* negative ones, however no statistically significant difference was found (P value > 0.05). Similarly, some studies also failed to find any general association between virulence markers and antibiotic resistance (18, 22, 37). This result is inconsistent with other studies that suggesting the statistically relation between drug resistance and *cagA*-positive strains (27, 31, 38, 39). There is little information about the relation between the variability of the strain and any outcome on the drug resistance rates of *H. pylori*. At the molecular level, there is no hypothetical basis for predicting an association between drug resistance and genotype; as such loci show to be neither physically nor functionally linked by genome organization (40).

Our research had some limitations. The studied population was not large enough, and our results need to be confirmed by studies on great number of patients. This study only tested the hypothesis that the *cagA* gene is related to antibiotic resistance. We think additional genetic or environmental factors might influence the interaction of *H. pylori* and antibiotics. In conclusion, the prevalence of *H. pylori* resistance is high in this area. Our results indicated that the relationship between drug resistance and the availability of *cagA* gene was not statistically significant. So, different treatment regimens are not recommended for patients with *H. pylori*- *cagA* positive infection.

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Authors' Contribution

None declared.

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References

1. Algood HM, Cover TL. Helicobacter pylori persistence: an overview of interactions between *H. pylori* and host immune defenses. *Clin Microbiol Rev*. 2006;**19**(4):597-613.
2. Rafeey M, Ghotaslou R, Nikvash S, Hafez AA. Primary resistance in Helicobacter pylori isolated in children from Iran. *J Infect Chemother*. 2007;**13**(5):291-5.
3. Erzin Y, Koksall V, Altun S, Dobrucali A, Aslan M, Erdamar S, et al. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA, babA2 genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. *Helicobacter*. 2006;**11**(6):574-80.
4. Rick JR, Goldman M, Semino-Mora C, Liu H, Olsen C, Rueda-Pedraza E, et al. In situ expression of *cagA* and risk of gastroduodenal disease in Helicobacter pylori-infected children. *J Pediatr Gastroenterol Nutr*. 2010;**50**(2):167-72.
5. Hatakeyama M, Higashi H. Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis. *Cancer Sci*. 2005;**96**(12):835-43.
6. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut*. 2007;**56**(6):772-81.
7. Chuah SK, Tsay FW, Hsu PI, Wu DC. A new look at anti-Helicobacter pylori therapy. *World J Gastroenterol*. 2011;**17**(35):3971-5.
8. van Doorn LJ, Schneeberger PM, Nouhan N, Plaisier AP, Quint WG, de Boer WA. Importance of Helicobacter pylori *cagA* and *vacA* status for the efficacy of antibiotic treatment. *Gut*. 2000;**46**(3):321-6.
9. Agudo S, Perez-Perez G, Alarcon T, Lopez-Brea M. High prevalence of clarithromycin-resistant Helicobacter pylori strains and risk factors associated with resistance in Madrid, Spain. *J Clin Microbiol*. 2010;**48**(10):3703-7.
10. Ryan KA, van Doorn LJ, Moran AP, Glennon M, Smith T, Maher M. Evaluation of clarithromycin resistance and *cagA* and *vacA* genotyping of Helicobacter pylori strains from the west of Ireland using line probe assays. *J Clin Microbiol*. 2001;**39**(5):1978-80.
11. McNulty C, Owen R, Tompkins D, Hawtin P, McColl K, Price A, et al. Helicobacter pylori susceptibility testing by disc diffusion. *J Antimicrob Chemother*. 2002;**49**(4):601-9.
12. Boyanova L, Stancheva I, Spassova Z, Katarov N, Mitov I, Koumanova R. Primary and combined resistance to four antimicrobial agents in Helicobacter pylori in Sofia, Bulgaria. *J Med Microbiol*. 2000;**49**(5):415-8.
13. Sambrook Joseph, Russell David W, Russell David W. Molecular cloning: a laboratory manual (3-volume set). 2001.
14. Ko JS, Kim KM, Oh YL, Seo JK. *cagA*, *vacA*, and *iceA* genotypes of Helicobacter pylori in Korean children. *Pediatr Int*. 2008;**50**(5):628-31.
15. Podzorski Raymond P, Podzorski Diane S, Wuerth Ann, Tolia Vasundhara. Analysis of the *vacA*, *cagA*, *cagE*, *iceA*, and *babA2* genes in Helicobacter pylori from sixty-one pediatric patients from the Midwestern United States. *Diagnos Microbiol Infect Dis*. 2003;**46**(2):83-88.
16. Basset C, Holton J, Gatta L, Ricci C, Bernabucci V, Liuzzi G, et al. Helicobacter pylori infection: anything new should we know? *Aliment Pharmacol Ther*. 2004;**20** Suppl 2:31-41.
17. Duck WM, Sobel J, Pruckler JM, Song Q, Swerdlow D, Friedman C, et al. Antimicrobial resistance incidence and risk factors among Helicobacter pylori-infected persons, United States. *Emerg Infect Dis*. 2004;**10**(6):1088-94.
18. De Francesco V, Margiotta M, Zullo A, Hassan C, Valle ND, Burattini O, et al. Clarithromycin resistance and Helicobacter pylori genotypes in Italy. *J Microbiol*. 2006;**44**(6):660-4.
19. Prazeres Magalhaes P, De Magalhaes Queiroz DM, Campos Barbosa DV, Aguiar Rocha G, Nogueira Mendes E, Santos A, et al. Helicobacter pylori primary resistance to metronidazole and clarithromycin in Brazil. *Antimicrob Agents Chemother*. 2002;**46**(6):2021-3.
20. Megraud F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. *Gut*. 2004;**53**(9):1374-84.
21. Dore MP, Piana A, Carta M, Atzei A, Are BM, Mura I, et al. Amoxicillin resistance is one reason for failure of amoxicillin-omeprazole treatment of Helicobacter pylori infection. *Aliment Pharmacol Ther*. 1998;**12**(7):635-9.
22. Godoy AP, Ribeiro ML, Benvenuto YH, Vitiello L, Miranda Mde C,

- Mendonca S, et al. Analysis of antimicrobial susceptibility and virulence factors in *Helicobacter pylori* clinical isolates. *BMC Gastroenterol.* 2003;**3**:20.
23. Mendonca S, Ecclissato C, Sartori MS, Godoy AP, Guerzoni RA, Degger M, et al. Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone in Brazil. *Helicobacter.* 2000;**5**(2):79-83.
 24. Milani Morteza, Ghotaslou Reza, Akhi Mohammad T, Nahaei Mohammad R, Hasani Alka, Somi Mohammad H, et al. The status of antimicrobial resistance of *Helicobacter pylori* in Eastern Azerbaijan, Iran: comparative study according to demographics. *J Infect Chemother.* 2012;**18**(6):848-852.
 25. Agudo S, Alarcon T, Cibrelus L, Urruzuno P, Martinez MJ, Lopez-Brea M. [High percentage of clarithromycin and metronidazole resistance in *Helicobacter pylori* clinical isolates obtained from Spanish children]. *Rev Esp Quimioter.* 2009;**22**(2):88-92.
 26. Lobo Gatti L, Agostinho Jn F, De Labio R, Balbo Piason F, Carlos Da Silva L, Fagundes De Queiroz V, et al. *Helicobacter pylori* and cagA and vacA gene status in children from Brazil with chronic gastritis. *Clin Exp Med.* 2003;**3**(3):166-72.
 27. Taneike I, Nami A, O'Connor A, Fitzgerald N, Murphy P, Qasim A, et al. Analysis of drug resistance and virulence-factor genotype of Irish *Helicobacter pylori* strains: is there any relationship between resistance to metronidazole and cagA status? *Aliment Pharmacol Ther.* 2009;**30**(7):784-90.
 28. Chomvarin C, Namwat W, Chaicumpar K, Mairiang P, Sangchan A, Sripa B, et al. Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. *Int J Infect Dis.* 2008;**12**(1):30-6.
 29. Ortiz-Princz Diana, Guariglia-Oropeza Verónica, Ávila Maira, Correnti María, Perrone Marianella, Gutierrez Beatriz, et al. *Helicobacter pylori* cagA and vacA genotypes in Cuban and Venezuelan populations. *Memórias do Instituto Oswaldo Cruz.* 2010;**105**(3):331-335.
 30. Ghasemian SH, Tavakoli H, Mojtahed JF, Salehi Rasoul SB, Pishva E. Correlation of cagA positive *Helicobacter pylori* Infection with clinical outcomes in Alzahra hospital, Isfahan, Iran. *J Res Med Sci.* 2008;**13**(4):196-201.
 31. Garcia Gabriella T, Aranda Katia RS, Gonçalves Manoel EP, Cardoso Silvia R, Iriya Kiyoshi, Silva Neusa P, et al. High prevalence of clarithromycin resistance and cagA, vacA, iceA2, and babA2 genotypes of *Helicobacter pylori* in Brazilian children. *J Clin Microbiol.* 2010;**48**(11):4266-4268.
 32. Tao R, Fang PC, Liu HY, Jiang YS, Chen J. A new subtype of 3' region of cagA gene in *Helicobacter pylori* strains isolated from Zhejiang Province in China. *World J Gastroenterol.* 2004;**10**(22):3284-8.
 33. Basso D, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, et al. Clinical relevance of *Helicobacter pylori* cagA and vacA gene polymorphisms. *Gastroenterology.* 2008;**135**(1):91-9.
 34. Broutet N, Marais A, Lamouliatte H, de Mascarel A, Samoyeau R, Salamon R, et al. cagA Status and eradication treatment outcome of anti-*Helicobacter pylori* triple therapies in patients with non-ulcer dyspepsia. *J Clin Microbiol.* 2001;**39**(4):1319-22.
 35. Karhukorpi J, Yan Y, Kolho KL, Rautelin H, Lahti M, Sirvio A, et al. cagA, vacA and iceA virulence genes of *Helicobacter pylori* isolates of children in Finland. *Eur J Clin Microbiol Infect Dis.* 2000;**19**(10):790-3.
 36. Hussein NR, Mohammadi M, Talebkhan Y, Doraghi M, Letley DP, Muhammad MK, et al. Differences in virulence markers between *Helicobacter pylori* strains from Iraq and those from Iran: potential importance of regional differences in *H. pylori*-associated disease. *J Clin Microbiol.* 2008;**46**(5):1774-9.
 37. Elviss NC, Owen RJ, Breathnach A, Palmer C, Shetty N. *Helicobacter pylori* antibiotic-resistance patterns and risk factors in adult dyspeptic patients from ethnically diverse populations in central and south London during 2000. *J Med Microbiol.* 2005;**54**(Pt 6):567-74.
 38. Mirodzhov GK, Ishankulova DM, Dustov AD. [The frequency and resistance of cytotoxin-associated (CAG-A) *Helicobacter pylori* strains to antibacterial agents in chronic gastritis and duodenal ulcer]. *Clin Med (Mosk).* 2006;**84**(5):51-4.
 39. Saruc M, Goksel G, Ozkaya S, Guclu F, Ozbakkaloglu B, Yuceyar H. The effect of CagA status on response to *Helicobacter pylori* eradication therapy in Western Turkey. *Braz J Med Biol Res.* 2001;**34**(11):1435-9.
 40. Elviss NC, Owen RJ, Xerry J, Walker AM, Davies K. *Helicobacter pylori* antibiotic resistance patterns and genotypes in adult dyspeptic patients from a regional population in North Wales. *J Antimicrob Chemother.* 2004;**54**(2):435-40.