



NON-INVASIVE MOLECULAR ANALYSIS OF *HELICOBACTER PYLORI* ISOLATED FROM ANTRAL BIOPSIES OF DYSPEPTIC PATIENTS

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ABSTRACT: This phenomenon of increased antibiotic resistance in infection management of *H.pylori* has grown in the last decade, inducing a dramatic decline in conventional regimen effectiveness. The causes of resistance are point mutations in bacterial DNA, which interfere with antibiotic mechanism of action, especially clarithromycin and levofloxacin. Therefore, international guidelines have recently discouraged their use in areas with a relevant resistance percentage, suggesting first-line schedules with expected high eradication rates, i.e., bismuth containing or non-bismuth quadruple therapies. These regimens require the daily assumption of a large number of tablets. Consequently, a complete adherence is expected only in subjects who may be motivated by the presence of major disorders. However, an incomplete adherence to antibiotic therapies may lead to resistance onset, since sub-inhibitory concentrations could stimulate the selection of resistant mutants. Of note, a recent meta-analysis suggests that susceptibility tests may be more useful for the choice of first than second-line or rescue treatment. Additionally, susceptibility guided therapy has been demonstrated to be highly effective and superior to empiric treatments by both meta-analyses and recent clinical studies. Conventional susceptibility test is represented by culture and antibiogram. However, the method is not available everywhere mainly for methodology-related factors and fails to detect hetero-resistances. Polymerase chain reaction (PCR)-based, culture-free techniques on gastric biopsy samples are accurate in finding even minimal traces of genotypic resistant strains and hetero-resistant status by the identification of specific point mutations. The need for an invasive endoscopic procedure has been the most important limit to their spread. A further step has, moreover, been the detection of point mutations in bacterial DNA fecal samples. Few studies on clarithromycin susceptibility have shown an overall high sensitivity and specificity when compared with culture or PCR on gastric biopsies. On these bases, two commercial tests are now available although they have shown some controversial findings. A novel PCR method showed a full concordance between tissue and stool results in a preliminary experience. In conclusion, despite poor validation, there is increasing evidence of a potential availability of noninvasive investigations able to detect *H. pylori* resistances to antibiotics. These kinds of analysis are currently at a very early phase of development and caution should be paid about their clinical application. Only further studies aimed to evaluate their sensitivity and specificity will afford novel data for solid considerations. Nevertheless, noninvasive molecular tests may improve patient compliance, time/ cost of infection management and therapeutic outcome. Moreover, the potential risk of a future increase of resistance to quadruple regimens as a consequence of their use on large scale and incomplete patient adherence could be avoided.

Key words: *Helicobacter pylori*; Antibiotic resistance; Non-invasive molecular test; Dyspeptic patients

INTRODUCTION: Recently, studies about *H. pylori* infection worldwide have shown some challenging truths: (1) bacterium spreading is still ongoing[1]; (2) for no other infection such a large number of therapeutic regimens has been proposed[2]; (3) the results are controversial: the same pattern can give exciting or disappointing results depending on geographical areas[3]; and (4) although many experts claim that there are no intractable but only inadequately treated *H. pylori* strains, currently no study in the world has displayed a 100% therapeutic success rate, i.e., the ideal therapy does not still exist[4]. It is undeniable that above listed problems are related to the matter of antibiotic resistances [5]. The magnitude of the phenomenon has grown in the last decade, thus both inducing a dramatic decline in the effectiveness of conventional treatment regimens and stimulating the exploration of the basis of antibiotic failure. Resistances may be divided into: (1) primary: present in subjects never treated for *H. pylori*; (2) secondary: acquired after one or more treatment schedules; and (3) hetero-resistance: coexistence of both susceptible and resistant strains in the same subject [6]. The causes of resistance are point mutations in bacterial DNA, which interfere with the mechanism of action of the different antibiotics. Clarithromycin, used for a long period as the key-antibiotic in many regimens, inhibits protein synthesis of the bacterium by acting at 23S ribosome subunit [7]. A dozen of point mutations in this site have been described worldwide. Interestingly, the main 23S DNA changes inducing clarithromycin resistance are dissimilar in Western and Eastern countries. These concerns depict a heterogeneous scenario of clarithromycin resistance in the different geographic areas [3]. On the other hand, levofloxacin mechanism of action involves the inhibition of gyrase enzyme with a consequent failure of bacterial DNA synthesis [6]. GyrA gene codifies for gyrase enzyme. Five-point mutations in this site, able to induce resistance to this antibiotic, have been observed [9].

Therapeutic Strategies: On these bases, key international guideline agencies have recently discouraged the use of clarithromycin and levofloxacin in areas with a resistance rate higher than 15% in first and second-line regimens, respectively [10,11]. Nevertheless, guidelines are designed for the clinical practice within an empirical setting, i.e., where susceptibility testing is unavailable, epidemiological data are taken into account to allow for the rational use of antibiotics. Therefore, suggested first line schedules are those with an expected high overall eradication rate, i.e., bismuth containing quadruple therapy or non-bismuth concomitant quadruple therapy [10,11] according to Maastricht V and Toronto guidelines. However, the 2009 Asia-Pacific guidelines suggest 14-d triple therapy or bismuth containing quadruple in first line, stating, moreover, that sequential therapy is not supported by convincing data in Asian countries [12]. On the other hand, the comparison of an old and outdated treatment vs a more recent and successful one should not put forward any doubt about the outcome.

Regimens require the daily assumption of a high number of tablets (14 and 8, respectively) [13]. An evident problem arising from this issue is related to the compliance of patients. A complete adherence is expected by subjects who may be motivated by the presence of major conditions, such as MALT lymphoma, family history of gastric cancer or peptic ulcer, particularly when complicated with episodes of bleeding, and the need for a concomitant long-term assumption of nonsteroidal anti-inflammatory drugs [13]. On the other hand, *H. pylori* positive dyspeptic or asymptomatic population probably may not be driven by similar motivations, given that in most cases the eradication of the

bacterium is not accompanied by a tangible clinical benefit [14]. However, an incomplete adherence to an antibiotic therapy may be an important reason of resistance onset, since sub-inhibitory concentrations in the vicinity of *H. pylori* could stimulate the selection of resistant mutants [15].

Bismuth containing quadruple therapy encloses the use of tetracycline, which, currently, shows very low resistance percentages in Europe with a trend to increase from West towards East (1%-5%) [3]. However, in Asia even tetracycline resistance rates of 19% are reported [16]. Therefore, an interesting question for the future could be represented by the risk of an increase of resistance to this antibiotic due to its use on a large scale and the incomplete patient adherence to its intake [17]. On the other hand, metronidazole resistance reaches high values worldwide especially in Asian regions, where it is generally estimated not lower than 50% [3]. No data regarding bismuth resistance has been described at the best of our knowledge.

Concomitant therapy implies the use of three conventional antibiotics with the evident aim to overcome the resistance to each single drug by the overall combined effect of the regimen. Presumably, for this reason, concomitant schedule is suggested as the treatment of choice, when compared to sequential therapy [18]. A final point concerns the duration of suggested first line therapies. Commercial kit of bismuth containing quadruple therapy provides a pill number necessary for a 10-d schedule and the same period is the minimal time requested for concomitant regimen. Nevertheless, a prolongation to 14-d of both treatments seems to improve their effectiveness [10,11].

Tailored Therapy: A meta-analysis by Wenzhen et al [19] carried out in 2010, suggested that first-line susceptibility guided triple therapy achieved a significantly higher *H. pylori* eradication than standard triple therapy. A successive similar analysis by López-Góngora et al [20] was performed in 2015 and included twelve studies, clearly demonstrating that susceptibility guided therapy was superior to empirical one. However, the same study also revealed no significant differences between second-line susceptibility-guided and empirical therapies. Moreover, the low number of studies and their high heterogeneity did not allow drawing any conclusion for rescue treatments, despite current guidelines recommend culture and antibiotic susceptibility test after first and second-line regimen failure. An interesting and original learning could emerge from the data of this last meta-analysis, i.e., susceptibility tests may be more likely useful for the choice of first than second-line or rescue treatment. Two recent studies performed in 2016 in Poland and China confirmed the excellent performance of tailored therapy as first line approach, a culture guided triple therapy in first line may achieve the 95.5% and 96.6% of success rate, at per protocol and intention to treat analysis, respectively [21]. Additionally, Zhou et al [22] demonstrated that a susceptibility-based treatment in first line achieved a gain of about 10% in eradication rate over empiric concomitant or triple plus bismuth regimens.

METHODS:

Invasive & Non-Invasive Susceptibility Tests: Conventional susceptibility test is represented by culture and antibiogram (E-test) in *H. pylori* isolates. Nevertheless, this investigation is recommended by current guidelines only after repeated treatment failures. The reasons hampering its widespread use are mainly due to relatively high rate of false negatives, often showing a low sensitivity [23].

Moreover, the method is not available everywhere mainly for methodology related factors (number of gastric biopsies/time consuming endoscopic procedures, conditions/interval of biopsy sample transport, laboratory characteristics and time needed for the result of the investigation). Of note, a failure of E-test in hetero-resistance detection has been observed [24]. On these bases, in the last years, different polymerase chain reaction (PCR)-based approaches have been developed as alternative tools to bacterium culture. These techniques allow assessing, on gastric biopsy samples, point mutations responsible for antibiotic resistance [25]. PCR-based, culture-free techniques are accurate in finding even minimal traces of genotypic resistant strains as well as in detecting hetero-resistant status [26]. In detail, in our experience a post-hoc subgroup study, enrolling 146 *H. pylori* positive patients and comparing real time (RT)-PCR (genotypic) and E-test on bacterial culture (phenotypic) for clarithromycin resistance analysis, showed an overall prevalence of clarithromycin phenotypic resistance significantly lower than genotypic one. A concordance of 71.2% between the two methods was found. This value of concordance may be due to three main factors: the relative low sensitivity of phenotypic investigation, its lack of hetero-resistance detection and the possibility that E-test may identify resistant strains with point mutations different from that tested by RT-PCR in the study. Molecular tests have been reported as promising approaches for resistance detection [26,27] even if they are not used in the clinical practice. The need for an invasive endoscopic procedure has been the most important limit to their spread. Therefore, many attempts have been performed in order to overcome this drawback.

RESULT & DISCUSSION: Since 2004, our group demonstrated the possibility of *H. pylori* DNA isolation from stool samples [28]. Successively, other evidences confirmed this diagnostic chance [29-31]. A further step was undoubtedly represented by the detection of point mutations conferring antibiotic resistance in bacterial DNA fecal samples. Table 1 reports the main studies on antibiotic susceptibility (clarithromycin) performed on *H. pylori* stool DNA by RT-PCR [32-38]. For each study, diagnostic accuracy parameter and reference standard are reported. An overall high sensitivity and specificity has been observed when fecal molecular tests have been compared with culture and RT-PCR on gastric biopsies. This kind of investigation is of particular interest in the pediatric population for the need of limiting the use of invasive procedures. In detail, two studies [35,38] have been performed in children. Booka et al [35] found a 91% of diagnostic accuracy in reference to *H. pylori* antigen stool and a 31% prevalence of clarithromycin resistance in Japanese children. A similar result was obtained by Vécsei et al [38] in Austria with a diagnostic accuracy of 90.2% for bacterium detection compared to rapid urease test and histology as well as of 94% for clarithromycin resistance compared to culture. On these bases two commercial tests are now available. A first noninvasive investigation using stool polymerase chain reaction (*H. pylori* ClariRes assay, Ingenetix, Vienna, Austria) analyzed the A2142G mutation for clarithromycin resistance [38]. However, the presence of the other two major mutations responsible for the resistance to this antibiotic in developed countries (A2143G and A2142C) was not shown directly, but only hypothesized on the basis of some RT-PCR cycle temperature parameters. Successively, another molecular commercial test was developed, i.e., the Genotype HelicoDR assay (Hain Lifescience GmbH, Nehren, Germany), which allows for the detection of molecular *H. pylori* resistances to clarithromycin and fluoroquinolones [39,40]. It

identifies both the most common point mutations (A2146C, A2146G and A2147G) for clarithromycin resistance and *gyrA* gene mutations located at positions 87 (N87K) and 91 (D91N, D91G, D91Y) for fluoroquinolone one. This investigation has been used in tissue samples and only recently applied to stool *H. pylori* DNA [41]. However, a low concordance between stool and biopsy samples for clarithromycin and fluoroquinolone resistance detection was found.

Table 1: Antibiotic susceptibility (clarithromycin): studies on *H. pylori* stool DNA by real time PCR

Ref.	Reference standard	Sensitivity	Specificity	Clarithromycin resistance
Scaletsky <i>et al</i> [32]	PCR on gastric biopsy	83.3%	100.0%	26.7%
¹ Vécsei <i>et al</i> [38]	Culture	89.2%	100.0%	45.1%
Noguchi <i>et al</i> [33]	Culture	NA	NA	20.4%
Rimbara <i>et al</i> [34]	Culture	96.6%	91.3%	13.3%
¹ Booka <i>et al</i> [35]	Culture	NA	NA	31.0%
Schabereiter-Gurtner <i>et al</i> [36]	Culture	98.0%	98.0%	24.4%
Fontana <i>et al</i> [37]	Culture	100.0%	100.0%	1.6%

over demonstrated that the presence of fecal material (300 mg) increased the amount of colony forming units (CFU)/mL required to obtain a positive result of the detection of bacterial DNA. In detail, a clear positivity was reached by a concentration of 1.5×10^6 CFU/mL of pure bacteria and 1.5×10^3 of stool-mixed organisms. At the moment, a double-blinded study involving an adequate patient sample size is ongoing with the purpose to establish sensitivity, specificity, positive and negative predictive values of this noninvasive technique.

At the same time of HelicoDR assay use on stool samples, we preliminarily experienced a novel RT-PCR method (THD Fecal Test, Italy) in order to investigate clarithromycin resistance mutations in bacterial stool DNA [42]. The procedure showed a full concordance between tissue and stool results in 52 consecutive patients at the first diagnosis of infection. We found A2143G mutation in 10 (19.2%), A2142G in 4 (7.7%) and A2142C in 5 (9.6%) patients with an interest, a preliminary experience “in vitro”

CONCLUSION: Despite the lack of a validation, there is increasing evidence of a potential future availability of noninvasive investigations able to detect *H. pylori* resistances to antibiotics, such as clarithromycin and quinolones, which have been commonly used until now. These techniques, when performed before a first-line therapy, could allow ascertaining a subgroup of strains still sensitive to these drugs and, therefore, patients benefiting from old regimens, whose administration has been at the moment discouraged by current guidelines. However, noninvasive molecular test is currently at a very early phase of development; therefore, cautions should be paid when discussing about their possible clinical applications. Only further studies aimed to evaluate sensitivity and specificity of molecular tests will afford novel data to make more solid considerations. Indeed, a noninvasive susceptibility test may achieve some potential advantages, thus improving patient compliance, time/cost of infection management and therapeutic outcome. Finally, the potential risk of a future increase of resistance to quadruple regimens, suggested in first line by guidelines, as a consequence of their use on a large scale and incomplete patient adherence could be avoided [16].

REFERENCES

1. **Eusebi LH**, Zagari RM, Bazzoli F. Epidemiology of *Helicobacter pylori* infection. *Helicobacter* 2014; 19 Suppl 1: 1-5.
2. **O'Connor A**, Gisbert JP, O'Morain C, Ladas S. Treatment of *Helicobacter pylori* Infection 2015. *Helicobacter* 2015; 20 Suppl 1: 54-61.
3. **Ierardi E**, Giorgio F, Losurdo G, Di Leo A, Principi M. How antibiotic resistances could change *Helicobacter pylori* treatment: A matter of geography? *World J Gastroenterol* 2013; 19: 8168-8180.
4. **Georgopoulos SD**, Papastergiou V, Karatapanis S. Current options for the treatment of *Helicobacter pylori*. *Expert Opin Pharmacother* 2013; 14: 211-223.
5. **De Francesco V**, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, Ierardi E, Zullo A. Worldwide *H. pylori* antibiotic resistance: a systematic review. *J Gastrointest Liver Dis* 2010; 19: 409-414.
6. **Mégraud F**. *H. pylori* antibiotic resistance: prevalence, importance, and advances in testing. *Gut* 2004; 53: 1374-1384.
7. **De Francesco V**, Margiotta M, Zullo A, Hassan C, Troiani L, Burattini O, Stella F, Di Leo A, Russo F, Marangi S, Monno R, Stoppino V, Morini S, Panella C, Ierardi E. Clarithromycin resistant genotypes and eradication of *Helicobacter pylori*. *Ann Intern Med* 2006; 144: 94-100.
8. **De Francesco V**, Zullo A, Giorgio F, Saracino I, Zaccaro C, Hassan C, Ierardi E, Di Leo A, Fiorini G, Castelli V, Lo Re G, Vaira D. Change of point mutations in *Helicobacter pylori* rRNA associated with clarithromycin resistance in Italy. *J Med Microbiol* 2014; 63: 453-457.
9. **Liou JM**, Chang CY, Sheng WH, Wang YC, Chen MJ, Lee YC, Hung HW, Chian H, Chang SC, Wu MS, Lin JT. Genotypic resistance in *Helicobacter pylori* strains correlates with susceptibility test and treatment outcomes after levofloxacin- and clarithromycin-based therapies. *Antimicrob Agents Chemother* 2011; 55: 1123-1129.
10. **Fallone CA**, Chiba N, van Zanten SV, Fischbach L, Gisbert JP, Hunt RH, Jones NL, Render C, Leontiadis GI, **Moayyedi P**, Marshall JK. The Toronto Consensus for the Treatment of *Helicobacter pylori* Infection in Adults. *Gastroenterology* 2016; 151: 51-69.
11. **Malfertheiner P**, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; 66: 6-30.
12. **Fock KM**, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, Lam SK, Xiao SD, Tan HJ, Wu CY, Jung HC, Hoang BH, Kachintorn U, Goh KL, Chiba T, Rani AA. Second Asia-Pacific Consensus Guidelines for *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2009; 24: 1587-1600.
13. **Lee M**, Kemp JA, Canning A, Egan C, Tataronis G, Farraye FA. A randomized controlled trial of an enhanced patient compliance program for *Helicobacter pylori* therapy. *Arch Intern Med* 1999; 159: 2312-2316.
14. **Zullo A**, Hassan C, De Francesco V, Repici A, Manta R, Tomao S, Annibale B, Vaira D. *Helicobacter pylori* and functional dyspepsia: an unsolved issue? *World J Gastroenterol* 2014; 20: 8957-8963.

15. **Megraud F**, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut 2013; 62: 34-42.
16. **Graham DY**, Lee SY. How to Effectively Use Bismuth Quadruple Therapy: The Good, the Bad, and the Ugly. Gastroenterol Clin North Am 2015; 44: 537-563.
17. **Graham DY**, Lee YC, Wu MS. Rational *Helicobacter pylori* therapy: evidence-based medicine rather than medicine-based evidence. Clin Gastroenterol Hepatol 2014; 12: 177-186.
18. **Losurdo G**, Giorgio F, Iannone A, Principi M, Barone M, Di Leo A, Ierardi E. Role of concomitant therapy for *Helicobacter pylori* eradication: A technical note. World J Gastroenterol 2016; 22: 8638-8640.
19. **Wenzhen Y**, Yumin L, Quanlin G, Kehu Y, Lei J, Donghai W, Lijuan Y. Is antimicrobial susceptibility testing necessary before first-line treatment for *Helicobacter pylori* infection? Meta-analysis of randomized controlled trials. Intern Med 2010; 49: 1103-1109.
20. **López-Góngora S**, Puig I, Calvet X, Villoria A, Baylina M, Muñoz N, Sanchez-Delgado J, Suarez D, García-Hernando V, Gisbert JP. Systematic review and meta-analysis: susceptibility-guided versus empirical antibiotic treatment for *Helicobacter pylori* infection. J Antimicrob Chemother 2015; 70: 2447-2455.
21. **Ferenc S**, Gnus J, Kościelna M, Kinda M, Yarka A, Stewart L, Witkiewicz W. High antibiotic resistance of *Helicobacter pylori* and its effect on tailored and empiric eradication of the organism in Lower Silesia, Poland. Helicobacter 2017; 22: [PMID: 27879042 DOI: 10.1111/hel.12365]
22. **Zhou L**, Zhang J, Song Z, He L, Li Y, Qian J, Bai P, Xue Y, Wang Y, Lin S. Tailored versus Triple plus Bismuth or Concomitant Therapy as Initial *Helicobacter pylori* Treatment: A Randomized Trial. Helicobacter 2016; 21: 91-99.
23. **Kjoller M**, Fischer A, Justesen T. Transport conditions and number of biopsies necessary for culture of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis 1991; 10: 166-167.
24. **Monno R**, Giorgio F, Carmine P, Soleo L, Cinquepalmi V, Ierardi E. *Helicobacter pylori* clarithromycin resistance detected by E-test and TaqMan real-time polymerase chain reaction: a comparative study. APMIS 2012; 120: 712-717.
25. **De Francesco V**, Margiotta M, Zullo A, Hassan C, Valle ND, Burattini O, Cea U, Stoppino G, Amoroso A, Stella F, Morini S, Panella C, Ierardi E. Primary clarithromycin resistance in Italy assessed on *Helicobacter pylori* DNA sequences by TaqMan real-time polymerase chain reaction. Aliment Pharmacol Ther 2006; 23: 429-435.
26. **De Francesco V**, Zullo A, Ierardi E, Giorgio F, Perna F, Hassan C, Morini S, Panella C, Vaira D. Phenotypic and genotypic *Helicobacter pylori* clarithromycin resistance and therapeutic outcome: benefits and limits. J Antimicrob Chemother 2010; 65: 327-332.
27. **Ierardi E**, Giorgio F, Losurdo G, Sorrentino C, Principi M, Di Leo A. Detection of *Helicobacter pylori* DNA sequences in gastric biopsy samples to refine the diagnosis and therapy. J Med Microbiol 2015; 64: 788-789.
28. **Notarnicola M**, Russo F, Cavallini A, Bianco M, Jirillo E, Pece S, Leoci C, Di Matteo G, Di Leo A. PCR identification of *Helicobacter pylori* in feces from patients with gastroduodenal pathology. Med Sci Res 1996; 24: 785-787.

29. **Gramley WA**, Asghar A, Frierson HF, Powell SM. Detection of *Helicobacter pylori* DNA in fecal samples from infected individuals. J Clin Microbiol 1999; 37: 2236-2240.
30. **Shuber AP**, Ascaño JJ, Boynton KA, Mitchell A, Frierson HF, El-Rifai W, Powell SM. Accurate, noninvasive detection of *Helicobacter pylori* DNA from stool samples: potential usefulness for monitoring treatment. J Clin Microbiol 2002; 40: 262-264.
31. **Wiśniewska M**, Nilsson HO, Bak-Romaniszyn L, Rechciński T, Bielański W, Płaneta-Małecka I, Płonka M, Konturek S, Wadström T, Rudnicka W, Chmiela M. Detection of specific *Helicobacter pylori* DNA and antigens in stool samples in dyspeptic patients and healthy subjects. Microbiol Immunol 2002; 46: 657-665.
32. **Scaletsky IC**, Aranda KR, Garcia GT, Gonçalves ME, Cardoso SR, Iriya K, Silva NP. Application of real-time PCR stool assay for *Helicobacter pylori* detection and clarithromycin susceptibility testing in Brazilian children. Helicobacter 2011; 16: 311-315.
33. **Noguchi N**, Rimbara E, Kato A, Tanaka A, Tokunaga K, Kawai T, Takahashi S, Sasatsu M. Detection of mixed clarithromycin resistant and -susceptible *Helicobacter pylori* using nested PCR and direct sequencing of DNA extracted from feces. J Med Microbiol 2007; 56: 1174-1180.
34. **Rimbara E**, Tamura R, Tanuma M, Noguchi N, Kawai T, Sasatsu M. Evaluation of clarithromycin resistance in *Helicobacter pylori* obtained from culture isolates, gastric juice, and feces. Helicobacter 2009; 14: 156-157.
35. **Booka M**, Okuda M, Shin K, Miyashiro E, Hayashi H, Yamauchi K, Tamura Y, Yoshikawa N. Polymerase chain reaction--restriction fragment length polymorphism analysis of clarithromycin-resistant *Helicobacter pylori* infection in children using stool sample. Helicobacter 2005; 10: 205-213.
36. **Schabereiter-Gurtner C**, Hirschl AM, Dragosics B, Hufnagl P, Puz S, Kovách Z, Rotter M, Makristathis A. Novel real-time PCR assay for detection of *Helicobacter pylori* infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. J Clin Microbiol 2004; 42: 4512-4518.
37. **Fontana C**, Favaro M, Pietroiusti A, Pistoia ES, Galante A, Favalli C. Detection of clarithromycin-resistant *Helicobacter pylori* in stool samples. J Clin Microbiol 2003; 41: 3636-3640.
38. **Vécsei A**, Innerhofer A, Binder C, Gizci H, Hammer K, Bruckdorfer A, Riedl S, Gadner H, Hirschl AM, Makristathis A. Stool polymerase chain reaction for *Helicobacter pylori* detection and clarithromycin susceptibility testing in children. Clin Gastroenterol Hepatol 2010; 8: 309-312.
39. **Smith SM**, O'Morain C, McNamara D. Antimicrobial susceptibility testing for *Helicobacter pylori* in times of increasing antibiotic resistance. World J Gastroenterol 2014; 20: 9912-9921.
40. **Mégraud F**, Bénéjat L, Ontsira Ngoyi EN, Lehours P. Molecular Approaches to Identify *Helicobacter pylori* Antimicrobial Resistance. Gastroenterol Clin North Am 2015; 44: 577-596.
41. **Brennan DE**, Omorogbe J, Hussey M, Tighe D, Holleran G, O' Morain C, Smith SM, McNamara D. Molecular detection of *Helicobacter pylori* antibiotic resistance in stool vs biopsy samples. World J Gastroenterol 2016; 22: 9214-9221.
42. **Giorgio F**, Ierardi E, Sorrentino C, Principi M, Barone M, Losurdo G, Iannone A, Giangaspero A, Monno R, Di Leo A. *Helicobacter pylori* DNA isolation in the stool: an essential pre-requisite for bacterial noninvasive molecular analysis. Scand J Gastroenterol 2016; 51: 1429-1432.