A new non-invasive tool for guiding *H. pylori* treatment

Erin Beckman, Ilaria Saracino, Giulia Fiorini, Courtney Clark, Vladimir Slepnev, Denise Patel, Clarissa Gomez, Reddy Ponaka, Vecheslav Elagin, Dino Vaira

1Department of Medical and Surgical Sciences, University of Bologna, Bologna, 40138 Italy
2Meridian Bioscience, Inc., Cincinnati, Ohio 45244 USA

Correspondence: Dino Vaira
E-mail: Berardino.vaira@unibo.it
Received: August 16, 2017
Published online: September 25, 2017

Current guidelines recommend that all patients with documented *H. pylori* infection should be treated with an appropriate antibacterial therapy. Clarithromycin-based regimens are commonly used as a first line therapy in *H. pylori* positive patients, however soon after the introduction of clarithromycin-based therapies, treatment failures associated with resistance to clarithromycin were reported. In this study, we evaluated the feasibility of using a non-invasive sample type, stool, to detect the presence of *H. pylori* by molecular methods while concurrently detecting mutations associated with the resistance to clarithromycin. The sensitivity for *H. pylori* detection by PCR was 93.8% compared to composite reference methods with gastric tissue biopsy. Out of 213 true positives that were sequenced, 36.2% showed point mutations associated with CLA resistance (A2142C, A2142G, A2143G). The correlation of sequencing and PCR was 98%. The correlation of sequencing and eradication of the infection was 86%. The results of this test may be used as a tool for guiding treatment of *H. pylori* infection.


Copyright: © 2017 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

*Helicobacter pylori* infection is a major cause of gastric ulcer disease and gastritis in humans and is a risk factor for the development of gastric cancer. Current guidelines recommend that all patients with documented *H. pylori* infection should be treated with an appropriate antibacterial therapy. Clarithromycin-based triple therapy, consisting of a proton pump inhibitor (PPI) and amoxicillin or metronidazole, is commonly used as a first line treatment; however, resistance of *H. pylori* to clarithromycin has been gradually increased worldwide. Soon after the introduction of clarithromycin-based therapies, treatment failures associated with resistance to clarithromycin were reported. Genetic analysis of resistant strains isolated from patients who failed primary clarithromycin-based treatment, identified mutations in the 23S ribosomal RNA (rRNA) as the predominant cause of resistance. In particular, point mutations in positions 2143 (A to G) and 2142 (A to G/C), are responsible for more than 90% of clarithromycin resistance cases. Moreover, the presence of the A2143G mutation is strongly associated with eradication therapy failure. A lower prevalence of A2142G/C mutation has been described, but these mutations are also commonly found in patients in whom clarithromycin-based treatment failed to eradicate the infection. A recent global consensus report on *H. pylori* gastritis gives a recommendation to assess the outcome of eradication therapy, preferably using a non-invasive test such as the stool antigen or urea breath test. In the case of...
eradication failure, there is a choice between several alternative treatments\(^5\). Testing for antibiotic sensitivity has been complicated for \textit{H. pylori} due to the necessity to perform culture and antimicrobial susceptibility test, which require additional biopsies and specific laboratory competence.

The approach of using a stool specimen in a molecular test for clarithromycin resistance has been proposed\(^1\). To date, there is very limited data on use of such approach in clinical research. It appears that, while the specificity of the molecular test has been consistently very high (more than 95%), the achievement of a high sensitivity remains very challenging\(^1\).

For this study, stool samples were acquired from a population of patients tested for \textit{H. pylori} infection in Bologna, Italy. Samples were collected from January 2015 to January 2016 and tested by PCR within a month of collection. Samples were frozen and kept at -20°C prior to testing. Patients were diagnosed as \textit{H. pylori} positive or negative according to concordant results of at least two positive tests of the composite reference method (CRM): histology, \(^{13}\text{C}-\text{urea breath test}, \text{rapid urease test}, \text{and/or culture alone}\(^1\). Total DNA was extracted from 294 raw stool samples using QIAamp FAST Stool Kit (QIAGen) and stored at -20°C prior to qPCR testing. TaqMan real-time PCR amplification using \textit{H. pylori} -specific reagents (Meridian Bioscience, Inc.) detected the presence of \textit{H. pylori} as well as predicted the phenotype of the organism and the related outcome of patients treated with clarithromycin. PCR performance in detecting the presence of \textit{H. pylori} was calculated based on CRM as the gold standard. The CLA resistance prediction due to the presence of 23S rRNA mutations was performed in parallel with detection of \textit{H. pylori}. Amplification observed in the green channel (Meridian Bioscience; Cat#ASR101) of the same qPCR result was further analyzed to make a prediction of CLA resistance or susceptibility.

Two alternative approaches were employed to analyze amplified DNA and make a determination of CLA susceptible or resistant for each sample. The first approach comprised of using differential fluorescent signal analysis and finding the derivate for both the green and red signal for every cycle. The difference between the green and red signal determined the resistance prediction. If the red signal had an attenuated result and, therefore, a positive delta from the green signal, the sample was called as containing mutation conferring clarithromycin resistance. If the red signal was equivalent to, in most cases, stronger than the signal in the green channel, the result would be a negative delta from the green signal. The sample was then deemed as absent of targeted mutations and susceptible to clarithromycin. The second approach to analyze the \textit{H. pylori} amplified target and make a prediction of resistance was by melt curve analysis in the temperature range of 52°C-72°C. When a single peak was observed at temperatures lower than 60°C, typically 54-56°C, the sample was deemed as containing mutations conferring resistance to clarithromycin. When a single peak was observed above 60°C, typically 63-65°C, the sample was deemed as lacking targeted mutations and susceptible to clarithromycin. Samples demonstrating amplification in green channel and no amplification in red channel were called as \textit{H. pylori} positive and indeterminate for the presence of clarithromycin-resistant mutations. The identity of amplified PCR products was confirmed by Sanger Sequencing.

PCR data were compared to the outcome of the eradication of the infection following clarithromycin combination treatment for a subset of patients. All patients underwent a standard UBT at the beginning and 4-6 weeks following the end of antibiotic therapy. The UBT was
performed after an overnight fast. Means and their 95% confidence intervals were calculated. Comparisons among patient subgroups were performed using the chi-square. Test accuracy was calculated with “2x2” table method (MEDCALC 17.2). A P level less than .05 was considered significant.

Out of 294 total stool samples from *H. pylori* patients, 93.8% (CI: 90-96.5%) (227/242) was the sensitivity in comparison with the CRM (excluding 2 invalid results). Because the current study targeted predominantly positive patient population in order to establish clinical sensitivity of the stool PCR method, we did not draw conclusions regarding clinical specificity. Twenty-six (26) samples had results that were not able to be evaluated either by PCR and/or sequencing for various reasons and were not included in final statistics. The CLA susceptibility prediction from PCR was verified by sequencing that established the identity of nucleotides in positions 2142 and 2143 of the 23S rRNA gene. Out of 213 remaining positive samples with an evaluable sequencing and PCR result, 77 (36.1%) contained mutations and 136 (63.8%) contained the wild type sequence. Among the mutations, 3 samples contained A2142C sequence, 19 samples contained A2142G sequence, and 55 samples contained A2143G sequence. One (1) sample that was predicted by PCR to be a wild type, contained a mutation at the A2143G position. PCR correctly identified 133 out of 136 samples (97.8%) containing wild type (CLA sensitive) sequence and 76 out of 77 (98.7%) samples containing mutations. This is summarized in Figure 1. Of 121 positive patients, treated by sequential or concomitant regimen that included clarithromycin, 100 were infected with the wild-type strain (82.6%) and 21 were infected with mutant strains (17.4%). A follow-up of the patients after treatment showed 93 of the wild-type *H. pylori* infected patients (93/100) were eradicated and 8 out of 21 mutant infected patients were not eradicated when treated with clarithromycin. The rate for each of these subsets is summarized in Figure 2. The correlation between genotype prediction by PCR and eradication of patients treated with clarithromycin was 83.5% (93+8/121). Additional 3 patient outcomes matched sequencing, for a final genotypic correlation of 86% (96+8/121). This assay can be used as a stand-alone screening tool since it can detect the presence of *H. pylori* as well as resistance, additionally as a reflex test once negative patients are eliminated by a stool antigen test or UBT. Both approaches often predict susceptibility to clarithromycin using non-invasive sample types as a tool to guide antibacterial treatment selection. This could improve cure rate of patients infected with *H. pylori* strains sensitive to clarithromycin and quickly move to an alternative regimen in cases of detection of resistant strains. There is a significant correlation between the presence of such mutations and the outcome of clarithromycin-based eradication treatment. There is also an extremely high rate (93%) of patients in the wild-type group to confirm that if a patient is infected with the sensitive strain, the outcome of eradication is highly favorable. There are various combination treatment regimens that include clarithromycin, this high rate included several of these, but we do not distinguish amongst them here.

In summary, these data demonstrate that *Helicobacter pylori* DNA can be detected in human stool specimens with a high sensitivity and, therefore, can be used to determine *H. pylori* presence with a similar performance as other direct
assays, such as SAT or UBT \[^{14}\]. More importantly, the genotypic resistance to clarithromycin can be detected without obtaining a biopsy sample in order to choose the right therapeutic approach. This finding confirms previous reports describing the use of PCR detection and assessment of clarithromycin sensitivity using stool specimens \[^{16}\]. The high analytical specificity and clinical sensitivity of this molecular \( H. pylori \) resistance assay means a strong likelihood of accurately predicting eradication for CLA therapy. This stool/PCR test has the potential to reduce healthcare costs with an updated algorithm. A biopsy sample requires the patient to return to the healthcare provider for an invasive procedure if treatment is unsuccessful \[^{3}\]. The PCR test option with the non-invasive sample can significantly reduce the need for biopsy samples currently used to determine resistance in patients who don’t need to undergo upper endoscopy according to clinical guidelines \[^{4}\].

References

15. EUCAST Clinical Breakpoint Table v. 5.0, valid from 2015-01-01. Available at: http://www.eucast.org/clinical_breakpoints/