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# Effect of metronidazole resistance on *Helicobacter pylori* eradication regimens

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**Objectives:** Guidelines suggest bismuth-containing quadruple therapy (BQT) or concomitant therapy (CT) as first-line therapy in our geographic area. Both schedules contain metronidazole. We aimed to evaluate the effect of metronidazole resistance to *Helicobacter pylori* (*H. pylori*) eradication therapy.

**Methods:** We recruited treatment-naïve subjects with *H. pylori* infection who received either CT or BQT during January 2020 and December 2021. Before therapy, a fecal sample was collected using the THD fecal test device from each patient. *H. pylori* DNA was extracted and mutations of *rdxA* and *frxA* genes and A2143G for metronidazole and clarithromycin resistance were investigated using real-time polymerase chain reaction with a high-resolution melting curve.

**Results:** Ninety-six patients were enrolled, including 29 received BQT and 67 received CT. The overall eradication rate was 94.8% (100% for BQT and 92.5% for CT). Metronidazole resistance was found in 18 (18.8%) subjects, while clarithromycin resistance was found in 19 (19.8%). All 18 patients with metronidazole resistance achieved successful eradication (five treated with BQT and 13 with CT). The eradication rate in metronidazole-sensitive strains was 93.6%. Of these, 24 received BQT with 100% success, and 54 had CT with five failures (successful eradication in 90.7%). Two patients with treatment failure were resistant to clarithromycin, and the remaining three were susceptible to both clarithromycin and metronidazole. No statistical significance was observed in the eradication rate between metronidazole-resistant and -sensitive strains (100% vs 93.6%, $P = 0.58$).

**Conclusion:** Metronidazole resistance does not influence the eradication rate of BQT and CT regimens in our geographical area, even if such results need to confirmed in a larger sample.

KEYWORDS
antibiotic resistance, bismuth-containing quadruple therapy, concomitant therapy, *Helicobacter pylori*, metronidazole

## 1 | INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a Gram-negative bacterium that colonizes the human stomach, thus causing several conditions including gastritis and peptic ulcer,[1] as well as extragastric disorders such as iron deficiency anemia, vitamin $B_{12}$ deficiency, and immune thrombocytopenic purpura.[2] Moreover, *H. pylori* has been recognized as a class I carcinogen due to its strong link with gastric adenocarcinoma. In addition, the organism may play a crucial role in the development of precancerous lesions such as atrophic gastritis and intestinal metaplasia.[3]

Eradication therapy for *H. pylori* infection includes the combination of antacids and two or more antibiotics. According to the guidelines, in our geographic area, which is characterized by a clarithromycin resistance rate of over 15%, bismuth-containing quadruple therapy (BQT) or concomitant therapy (CT) is recommended as the first-line therapy.[4] CT consists of a combination of amoxicillin, clarithromycin, and metronidazole with a proton pump inhibitor (PPI) for 10 days. BQT is nowadays given as a "three in one" formulation, that is, a capsule containing metronidazole, tetracycline, and bismuth subcitrate, plus a PPI. Both regimens have shown excellent results, with an eradication rate close to 90%.[5–7] Resistance to clarithromycin has been largely investigated and is regarded as the most relevant factor against successful eradication.[8]

On the other hand, resistance to metronidazole has been less analyzed, even if some reports show that the resistant rate may be over 60% in some geographical areas such as Southeastern Asia.[9] It is a common opinion that a double clarithromycin–metronidazole resistance may undermine the efficacy of CT, thus recommending BQT in these cases.[10] The mechanism of resistance to metronidazole is not as straightforward. Alteration of *rdxA* gene is the most relevant cause of drug resistance, even if a panel of point mutations rather than a single mutation could contribute to inducing the phenomenon.[11] Moreover, other genes such as *frxA* also seem to be involved.[12]

Based on such premises, we aimed to investigate how resistance to metronidazole may impact eradication rate of regimens containing this antibiotic, namely CT and BQT, in treatment-naïve patients with *H. pylori* infection.

## 2 | PATIENTS AND METHODS

### 2.1 | Patient recruitment

We prospectively recruited consecutive treatment-naïve patients with *H. pylori* infection during January 2020 and December 2021. The primary outcome was to investigate how metronidazole resistance might impact *H. pylori* eradication rate by using the regimens containing this antibiotic. All patients underwent gastroscopy with gastric mucosal biopsy (two biopsy samples from the antrum and two from the corpus), rapid urease test (RUT), and $^{13}$C-urea breath test (UBT). In detail, for this last investigation, an isotope ratio mass spectrometer (ABanalitica, Padua, Italy) was used with 75 g urea and 1.4 g citric acid. For RUT an in-house made kit was used, as described in the previous study.[13] *H. pylori* infection was confirmed when two or more abovementioned tests obtained positive results. For each patient, the following data were collected: age, sex, main symptoms prompting gastroenterological examination, comorbidities, and medications consumed.

Exclusion criteria were: (a) age <18 years; (b) a previous history of gastric cancer; (c) unwillingness to participate in the study, or with psychiatric disorders or other conditions that were unable to provide a valid informed consent; (d) allergy to any drugs used for *H. pylori* eradication therapy; and (e) had been treated with PPIs or histamine $H_2$ receptor antagonists ($H_2$RAs) within 2 weeks prior to their enrollment, or with antibiotics or bismuth salts within 4 weeks before their enrollment. Additionally, patients with chronic diarrhea were also excluded from the study because of the difficulty in the collection of fecal samples.

This study was conducted in agreement with the Declaration of Helsinki (Brazil, 2013), and was approved by the Local Ethics Committee of AOU Consorziale Policlinico di Bari (protocol no. 74413, approved on 16 November 2016). This study was registered on ClinicalTrials.gov (NCT04107194). Written informed consent was obtained from each patient.

### 2.2 | *H. pylori* eradication therapy

Recruited patients received either CT or BQT, both lasting 10 days.[14] CT consisted of pantoprazole 40 mg twice daily, clarithromycin 500 mg twice daily, metronidazole 500 mg twice daily, and amoxicillin 1000 mg twice daily. BQT consisted of pantoprazole 40 mg twice daily and three capsules of Pylera (tetracycline 125 mg, metronidazole 125 mg, bismuth subcitrate potassium 140 mg per capsule) four times daily. Pantoprazole was administered 30 min before meals, while antibiotics were given after the main meals. Treatment was blindly prescribed by computer assignment regardless of molecular investigation result (post-hoc analysis). CT or BQT was chosen by the physician, and was taken in agreement with the patients. Adverse events and patient compliance were evaluated by a personal interview approximately 1 week after the treatment. Good compliance was defined as taking at least 90% of the prescribed pills.

*H. pylori* eradication was evaluated at least 6 weeks after the end of eradication treatment by $^{13}$C-UBT, and successful treatment was deemed if the test was negative.

### 2.3 | Evaluation of antibiotic resistance

A stool sample was collected from all patients using the THD Fecal Test Device (THD Spa, Correggio, Reggio Emilia, Italy). This device has a filter blocking real-time polymerase chain reaction (PCR), inhibiting substances like hemoglobin and its degradation products, polysaccharide complexes, heavy metals, and proteins. Furthermore, it eliminates large molecules such as fibers. The treated solution was finally taken

from the reservoir and processed for DNA extraction by QIAamp DNA Stool Minikit (Qiagen, Hilden, Germany). After this last phase, real-time PCR was performed to assess point mutations linked to *H. pylori* resistance to clarithromycin[15–18] and metronidazole, as previously described, and was extensively employed and validated. For metronidazole resistance, mutations of the *rdxA* and *frxA* genes were investigated. Real-time PCR followed by high resolution melting (HRM) was set to detect mutations. Curves produced by HRM were compared to those derived for wild-type, nonmutated genes from susceptible strains of 23S rRNA, *frxA*, and *rdxA* to detect resistances.[19] Control strains of mutated genes obtained from our biobank were also kept.

### 2.4 | Statistical analysis

Continuous variables were expressed as mean ± standard deviation, whereas discrete variables were expressed as numbers and proportions or percentages with 95% confidence interval (CI). Fisher's exact test and Student's *t*-test were used to compare differences in discrete and continuous variables, respectively. The eradication rate of *H. pylori* was estimated both as intention-to-treat (ITT) and per-protocol (PP) analyses. $P < 0.05$ was regarded as statistical significance. Statistical analyses were performed using the SPSS Statistics v.23.0 for Windows (IBM, Armonk, NY, USA).

## 3 | RESULTS

### 3.1 | Characteristics of the patients

Ninety-six consecutive treatment-naïve patients at a mean age of 56.1 ± 18.4 years with *H. pylori* infection were recruited, including 31 men and 65 women. Main symptoms of the patients were postprandial fullness (n = 18, 18.8%), early satiety (n = 12, 12.5%), epigastric pain (n = 25, 26.0%) and heartburn (n = 26, 27.1%). Among the most relevant comorbidities, seven (7.3%) had hypertension and three (3.1%) had diabetes mellitus. Overall, seven patients were excluded from the study, including one patient with a previous history of gastric cancer, three patients who refused to participate in the study, and three with allergy to antibiotics used in the study. Demographic and clinical characteristics of the patients are summarized in Table 1.

At endoscopic examination, gastric mucosal hyperemia was observed in 17 (17.7%) patients, while antral erosion was found in 18 (18.8%). Gastric and duodenal ulcers were found only in one (1.0%) and two (2.1%) patients, respectively. At histological analysis, active chronic gastritis was observed in all patients, which involved the antrum in 74 (77.1%) and the whole stomach in 22 (22.9%).

### 3.2 | Antibiotic resistance and eradication therapy

Altogether 29 and 67 patients received 10-day BQT and CT, respectively. No dropouts were observed; therefore, ITT and PP analyses were identical. The overall eradication rate was 94.8% (91/96; 95% CI 90.4%–99.2%), including 100% (29/29) for BQT and 92.5% (62/67; 95% CI 86.2%–98.8%) for CT, and there was no statistically significant difference between the two treatment regimens ($P = 0.53$). All patients had a good compliance by consuming over 90% of the medications. Adverse events were recorded in 15 (15.6%; 95% CI 11.9%–19.3%) patients, including abdominal pain or bloating in nine patients, diarrhea in one case, nausea in two cases, weakness or dizziness in two cases, and diarrhea and headache in one each, all of which were mild and recovered spontaneously.

Metronidazole resistance was detected in 18 (18.8%; 95% CI 11.0%–26.6%) patients, while clarithromycin resistance was seen in 19 (19.8%; 95% CI 11.9%–27.7%). Figure 1 shows the melting curves of patients and controls with wild-type *frxA* and mutated *frxA*.

All 18 patients with metronidazole resistance received *H. pylori* eradication therapy, including five received BQT and 13 received CT, respectively. Moreover, we detected four cases with combined double resistance, all of whom achieved successful eradication with BQT.

On the other hand, the eradication rate of metronidazole-sensitive strain was 93.6% (73/78; 95% CI 88.2%–99.0%). Of these, 24 received BQT, with 100% success. Out of the 54 patients who

**TABLE 1** Main demographic and clinical characteristics of the enrolled patients

| Variables | Patients (n = 96) |
|---|---|
| Sex (male/female) (n) | 31/65 |
| Age, years (mean ± SD) | 56.1 ± 18.4 |
| Symptoms (n, %) | |
| Postprandial fullness | 18 (18.8) |
| Early satiety | 12 (12.5) |
| Epigastric pain | 25 (26.0) |
| Heartburn | 26 (27.1) |
| Asymptomatic | 15 (15.6) |
| Previous antibiotics exposure (n, %) | |
| Amoxicillin | 10 (10.4) |
| Main comorbidities (n, %) | |
| Hypertension | 7 (7.3) |
| Diabetes mellitus | 3 (3.1) |
| Chronic kidney disease | 2 (2.1) |
| Benign prostatic hypertrophy | 2 (2.1) |
| Hashimoto thyroiditis | 1 (1.0) |
| Lung emphysema | 1 (1.0) |
| Endoscopic findings (n, %) | |
| Gastric hyperemia | 17 (17.7) |
| Antral erosions | 18 (18.8) |
| Gastric ulcer | 1 (1.0) |
| Duodenal ulcer | 2 (2.1) |
| Normal | 58 (60.4) |

Abbreviations: SD, standard deviation.

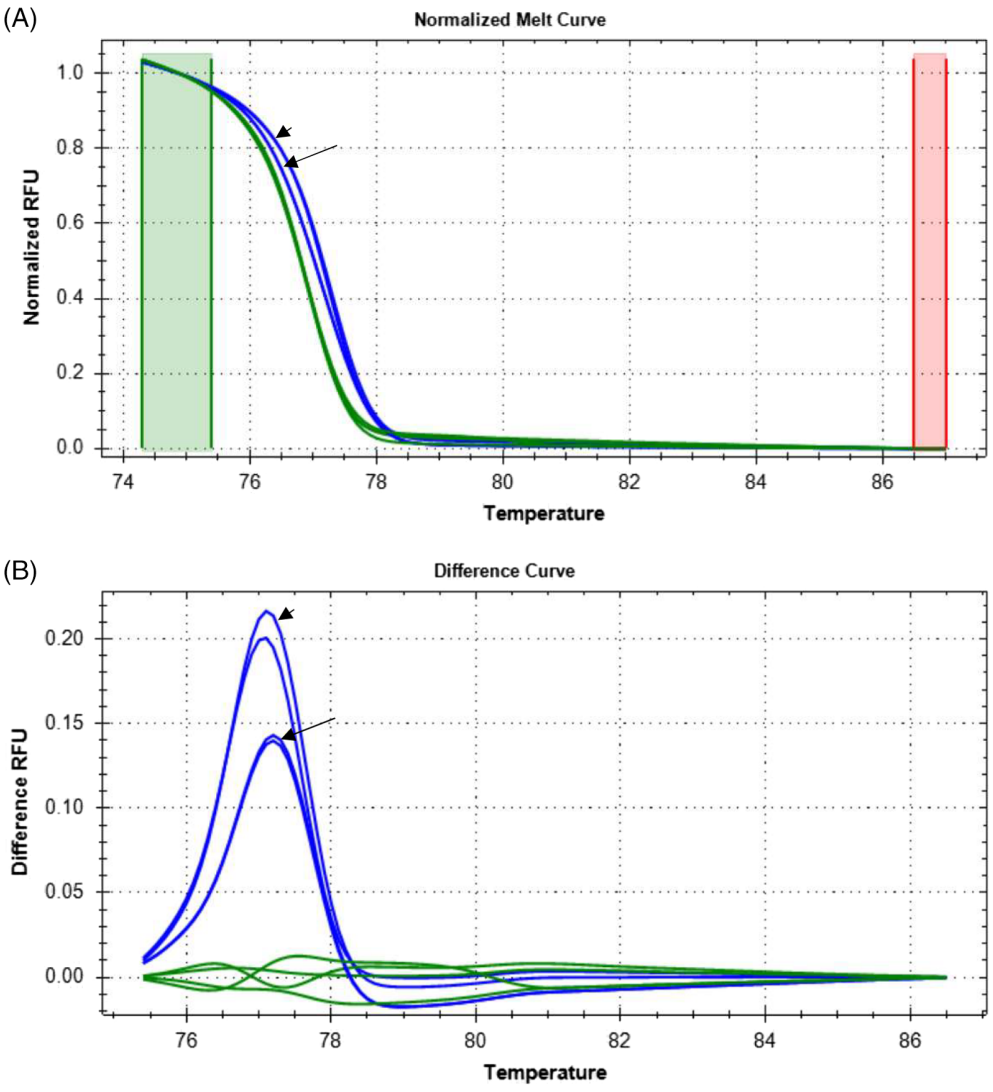


**FIGURE 1** Melting curves for *frxA* gene as reported as A, the normalized melt curve and B, difference curve. The green curves represent a control wild-type and a patient with wild-type gene, clustering together. The blue curves correspond to mutated genes; in particular, arrowhead indicates a mutated control and the arrow highlights a clustered mutated patient. Abbreviation: RFU, relative fluorescence unit


**FIGURE 2** Flowchart of the study. Abbreviations: BQT, bismuth-containing quadruple therapy; CT, concomitant therapy; CLA-R, clarithromycin-resistant; CLA-S, clarithromycin-susceptible; MET-R, metronidazole-resistant; MET-S, metronidazole-susceptible.

received CT, we observed five failures; among them, two patients were resistant to clarithromycin and the remaining three were susceptible to both clarithromycin and metronidazole.

The eradication rate of clarithromycin-resistant strains was 89.5% (17/19), while it was 96.1% (74/77) in susceptible strains, with no significant difference ($P = 0.25$).

Additionally, we failed to find any statistical significance in the eradication rate between metronidazole-resistant and -sensitive strains (100% vs 93.6%, $P = 0.58$). The main results are summarized in Figure 2.

## 4 | DISCUSSION

Antibiotic resistance is the leading problem in *H. pylori* eradication. While for clarithromycin, genetic background underlining the phenomenon has already been elucidated, with *A2143G*, *A2142G*, and *A2142C* point mutations being the most common reasons for resistance, few data are available for the characterization of metronidazole genotypic resistance. Despite *rdxA* and *frxA* being the genes that are more often involved in resistance mechanisms, a specific mutation typifying resistance has not been reported yet; therefore, it is presumable that a panel of point mutations could be involved.[11] On the other hand, some authors suggested that mutations in *rdxA* may not always be essential for metronidazole resistance.[20]

The effect of metronidazole resistance on eradication regimens is still a matter of debate. Long et al[21] found that the eradication rate of metronidazole-resistant and susceptible strains was 68.4% and 100%, respectively, in patients receiving a triple therapy with clarithromycin and metronidazole. Moreover, this study seemed to be in agreement with the observation that a previous exposure to metronidazole may be a risk factor for the failure of regimens containing this antibiotic.[22] Conversely, in a group of patients consuming a triple therapy with amoxicillin and metronidazole, the eradication rate was 97% and 82% in sensitive and resistant strains, respectively.[23] Similarly, patients with resistance to metronidazole received either a triple regimen with clarithromycin and metronidazole or a quadruple with bismuth, clarithromycin, and metronidazole, achieving an eradication rate of 85% and 94%, respectively.[24] The results of the last two reports are not significantly affected by metronidazole resistance, which are consistent with the findings of the present study.

Metronidazole is a nitroimidazole agent that may play a secondary role compared to other antibiotics such as clarithromycin or tetracycline for the treatment of *H. pylori* infection. However, its combination with the cited antimicrobials is essential, as it dramatically improves eradication rates.[25,26] Indeed, in areas with high resistance to metronidazole, the effectiveness of sequential therapy is low,[27] thus suggesting that double clarithromycin–metronidazole resistance could be regarded as the leading cause for the failure of this regimen.[28] Compared to sequential therapy, concomitant therapy implies a superior total amount of metronidazole to be consumed daily (1000 mg daily for 10 days instead of 5 days), and even a higher dose (1500 mg per day for 10 days) for BQT. Therefore, it is possible that increasing the metronidazole dose may overcome antibiotic resistance.[29–31] This standpoint presumably explains our finding, that is, the marginal effect of metronidazole resistance in affecting therapeutic outcome. Another possible explanation is that HRM is able to distinguish every mutation different from wild-type *rdxA* and *frxA* genes, but it is not able to determine whether these mutations could mirror a phenotypical resistant strain. Additionally, HRM may detect all genotypic variants, not only those which confer phenotypic resistance, as mentioned above, as well as heteroresistance. In this regard, previous studies from our group largely investigated these aspects.[32,33] This stimulates further investigations, comparing the clinical impact of genotypic and phenotypic metronidazole resistance. Another limitation may be due to the fact that the proportion between assigned therapies BQT and CT was 1:2. This happened because physicians were free to choose between them and, maybe, in our center a preference for CT was observed, which was probably due to a better compliance to a lower number of daily pills. The unbalanced proportion of BQT/CT regimens could be a bias. Another limitation could be the small sample size ($n = 96$) and the fact that this is a bicenter study, but the present study is only a preliminary experience and further results are expected. However, our data failed to show any difference in the eradication rate and antibiotic resistance between the two regimens, and that CT had a slightly worse effectiveness than BQT. A prolongation of treatment duration to 14 days could be beneficial in areas where effectiveness started to decline, as described by Yadollahi et al.[34] Otherwise, 14-day CT has not been investigated in depth, and most reports described an effectiveness similar to that reported in our study.[35] Such an approach has been already applied to sequential therapy to find a minimal, not statistically significant, increase in the eradication rate.[36]

Genotypic resistance investigation has some undoubted benefits. Additionally, the possibility of genotyping antibiotic resistance in stools by a noninvasive method is further attractive.[37–39] Indeed, this approach could become the future of antimicrobial treatment for *H. pylori*, since a tailored therapy, suggested in the first line, may be the most appealing and may help select the "fittest" antibiotic combination, thus avoiding both drug misuse and secondary resistance.[40,41]

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### CONFLICT OF INTEREST

A. Di Leo served as a consultant for THD SpA.

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