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Triple Therapy with High-Dose Proton-Pump Inhibitor, Amoxicillin, and Doxycycline Is Useless for *Helicobacter pylori* Eradication: A Proof-of-Concept Study

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Abstract

Introduction: *Helicobacter pylori* resistance to antibiotics is steadily increasing and multidrug-resistant strains are common and difficult to eliminate, mainly in countries where bismuth, tetracycline, furazolidone, and rifabutin are unavailable.

Aim: To evaluate the efficacy and safety of a triple therapy with proton-pump inhibitor (PPI), amoxicillin, and doxycycline in patients with multidrug-resistant *H. pylori*.

Patients and Methods: This prospective study involved 16 patients (13 females; mean age – 50 ± 11.3 years) infected by *H. pylori* with known resistance to clarithromycin, metronidazole, and levofloxacin, but susceptibility to amoxicillin and tetracycline. All patients were previously submitted to upper endoscopy with gastric biopsies for *H. pylori* culture and susceptibility testing by Etest. Mutations in 23S rRNA and gyrA genes were determined by real-time PCR. A 10-day eradication regimen with PPI (double-standard dose b.i.d.), amoxicillin (1000 mg b.i.d.), and doxycycline (100 mg b.i.d.) was prescribed after pretreatment with PPI during 3 days. Eradication success was assessed by ^{13}C-urea breath test 6–10 weeks after treatment. Compliance and adverse events were determined through phone contact immediately after treatment and specific written questionnaires.

Results: Only one patient did not complete treatment due to adverse events. Another four patients experienced mild side effects not affecting compliance. The control ^{13}C-urea breath test was positive in all patients. Per-protocol and intention-to-treat eradication rates were 0%.

Conclusions: Although safe, a triple-therapy protocol with high-dose PPI, amoxicillin, and doxycycline is useless for multidrug-resistant *H. pylori* eradication.

Helicobacter pylori (*H. pylori*) infection is a recognized worldwide problem affecting an estimated 50% of the global population [1,2]. *H. pylori* is associated with multiple gastric pathologies, including gastritis, gastroduodenal ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma [3].

There are specific indications for *H. pylori* eradication, and the empiric triple treatment comprising proton-pump inhibitors (PPIs), clarithromycin, and amoxicillin or metronidazole is universally accepted since it was proposed at the first Maastricht conference [2,4]. However, the efficacy of standard triple therapy has decreased in the last decades and is now inferior to 80% in several countries [2,5]. This is related to increased antibiotic consumption and increased resistance of *H. pylori* to clarithromycin [6]. When empiric treatments fail, the remaining options become more limited because *H. pylori* can acquire resistance to some antibiotics, specially clarithromycin, levofloxacin, and metronidazole,

which no longer should be used in subsequent treatments. Metronidazole is probably the exception because resistance to this drug can be partially overcome by increased dose and duration of treatment [7]. Strains of *H. pylori* that are resistant to at least two antibiotics are defined as multidrug resistant [8].

H. pylori is naturally not susceptible to multiple antibiotics including vancomycin, trimethoprim, sulfonamides, glycopeptides, cefsulodin and polymyxins [9,10]. Besides amoxicillin, clarithromycin, and metronidazole, the other antibiotics that can be used in *H. pylori* eradication are tetracyclines, fluoroquinolones (mainly levofloxacin), rifampins, fosfomycin, other 5-nitroimidazoles, and nitrofurans [9].

Doxycycline is a tetracycline analogue and may be an alternative. This antibiotic has bacteriostatic properties through the inhibition of bacterial protein synthesis. "In vitro" susceptibility of *H. pylori* to doxycycline had already been demonstrated, and different treatments with this antibiotic were previously presented but the results were heterogeneous [8,11–20].

Although it is possible to achieve a 99.5% cumulative *H. pylori* eradication success with an overall strategy of sequential empiric therapies, it is still recommended to perform gastric biopsies and antibiotic susceptibility testing after failure of two successive treatments [2,21]. Unfortunately, there are countries where multiple second- or third-line drugs currently used for *H. pylori* treatment are unavailable. Multiple resistant strains are almost untreatable in such circumstances and adapted therapies, even including doxycycline, are acceptable alternatives.

The aim of this study was to evaluate the efficacy and tolerability of a doxycycline-containing triple therapy in patients infected by *H. pylori* strains with known resistance to clarithromycin, metronidazole, and levofloxacin, but susceptibility to amoxicillin and tetracycline.

Patients and Methods

Patients

In this single-center study, consecutive patients infected by *H. pylori* with known resistance to clarithromycin, metronidazole and levofloxacin, but susceptibility to amoxicillin and tetracycline, were prospectively included. Exclusion criteria were: age under 18 years; pregnancy; lactating and/or fertile women who were not using safe contraceptive methods; history of allergy/hypersensitivity to any antibiotic or PPI; previous gastric malignancy and/or gastric surgery; current use of anticoagulants; marked thrombocytopenia; systemic severe disease (hepatic, cardiorespiratory or renal disease; uncontrolled diabetes; active malignant diseases, coagulopathies); use of antibiotics in the last 4 weeks; use of PPI in the last 2 weeks.

For all patients, prior eradication treatments and antibiotic consumption in the last 12 months were recorded. Information about age, gender, ethnic background, body mass index, local of residence, education level, alcohol and tobacco consumption, and history of frequent infections was also obtained and registered.

Following previous recommendations, this proof-of-concept study was designed for initial inclusion of 30 patients and would stop if a cure rate of 97% was achieved or if six failures occurred [22].

Study Design

All patients have previously received one or more failed eradication attempts. They were then submitted to upper endoscopy with biopsies in the antrum and the corpus that were immediately placed in independent containers of adequate transport media – Portagerm pylori (bioMérieux, Portugal) – at 4 °C, and sent to microbiology laboratory. Urease test and Gram staining of a smear prepared from the biopsy specimen were performed to confirm the presence of *H. pylori*. After manual grinding with disposable material, the samples were distributed directly in agar pylori (bioMérieux Portugal, Linda-A-Velha, Portugal). Cultures were incubated for a minimum of 72 hours and a maximum of 10 days at 37 °C under microaerophilic conditions, produced with H_2-CO_2-generating packs (GENbag; bioMérieux, Portugal). *H. pylori* isolates were identified by colony morphology, characteristic spiral morphology on Gram staining, and positive catalase, urease, and oxidase tests.

Minimum inhibitory concentrations (MIC) for amoxicillin, clarithromycin, metronidazole, levofloxacin, and tetracycline were determined by Etest (bioMérieux, Portugal). In order to minimize variations in the results, Hp ATCC 43504 strain was used for quality control of the susceptibility assay and a different microbiologist repeated all tests. MIC values were expressed in μg/mL, and strains were considered resistant to amoxicillin, clarithromycin, metronidazole, levofloxacin, and tetracycline at MIC >0.5, >1, >8, >1, and >1 μg/mL, respectively. MIC values were established according to the data available in 2009, including the CLSI breakpoints [9,23–28].

DNA extraction from pure culture of *H. pylori* was performed with a special extraction kit (QIAamp® DNA Mini Kits; QIAGEN, Izasa Portugal, Carnaxide, Portugal) according to the manufacturer's instructions. Point mutations in 23S rRNA gene, conferring resistance to

clarithromycin, and in the quinolone resistance-determining region of the gyrA gene, mainly involving amino acid substitutions at amino acid 87 and amino acid 91, were detected by real-time PCR using a LightCycler device, as previously described [25,29].

Eradication Therapy and Efficacy Assessment

All patients were prescribed with the following treatment: pantoprazole (80 mg b.i.d) for the first 3 days followed by a 10-day eradication regimen with pantoprazole (80 mg b.i.d.), amoxicillin (1000 mg b.i.d.), and doxycycline (100 mg b.i.d.).

After detailed explanation of the therapy and potential secondary effects to the patients on the day of clinical observation, they were given a diary to record all administrations, side effects, and symptoms during therapy. Patients were contacted by phone immediately after treatment to register the compliance as well as all potential symptoms or adverse events. They were submitted to ^{13}C-urea breath test (UBT) 6–10 weeks after ending the treatment, to assess *H. pylori* status. At this time, the compliance was, once more, confirmed by counting the tablets returned by the patient. Poor compliance was assumed if <80% of the drugs were taken.

The patients also returned the diaries fulfilled during treatment, and they were asked to assess the tolerance and efficacy of this therapeutic regimen in symptomatic improvement through visual scales from 0 to 10 (0 – no tolerance at all and 10 – excellent tolerance; 0 – no efficacy and 10 – full efficacy). Adverse events were scored as mild, moderate, or severe according to their influence in daily activities (daily activities not limited, limited in some extent, or not possible at all, respectively) and the need to discontinue treatment. The scoring system was based on the one proposed by de Boer et al. [30], with minor modifications. All along the study the patients had direct telephonic access to an investigator, in order to resolve all doubts and problems that eventually occurred.

No antibiotics or antisecretory drugs were allowed, 4 and 2 weeks, respectively, before the UBT. It was considered positive if the δ-value over baseline was ≥4‰.

Statistics

Categorical variables were presented with their relative and absolute values, and quantitative ones were expressed as mean ± standard deviation. Analysis of *H. pylori* eradication success was performed on an intention-to-treat (includes all eligible patients enrolled in the study, regardless of compliance with the study protocol; patients with unavailable data are assumed to have been unsuccessfully treated) and per-protocol (includes only patients with good compliance and with all data evaluable at the end of treatment) basis. If possible, univariate and multivariate analyses would be performed to evaluate the association of different variables with effectiveness of treatment.

Ethical Considerations

The study was approved by the ethical committee of our hospital and the Faculty of Medicine and performed in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice Guidelines, and applicable local laws and regulations. Signed informed consent was obtained from each patient.

Results

Sixteen patients, including 13 women and three men, with an average age of 50 years, were included. Demographic and clinical characteristics of these patients are presented in Table 1. The mean number of eradication attempts was 2.8 ± 1.3 (range: 1–6), and the drugs used during these treatments are summarized in Table 2.

Table 1 Demographic and clinical characteristics of the patients

Variables	n = 16
Mean age (years)	50 ± 11.3 (range 36–78)
Gender (%)	
Male	3 (18.8)
Female	13 (81.2)
Ethnic background (%)	
European	16 (100)
Residence (%)	
Rural	8 (50)
Urban	8 (50)
Indication(s) for *H. pylori* eradication (%)	
Dyspepsia	12 (75)
GERD/chronic therapy with PPI	4 (25)
First-degree relatives with gastric cancer	4 (25)
Iron-deficient anemia	2 (12.5)
Peptic ulcer	1 (6.3)
BMI (Kg/m^2)	27.2 ± 3.9 (range 19.6–33.8)
Smoking (%)	5 (31.3)
Alcohol consumption (%)	4 (25)
History of frequent infections (%)	2 (12.5)
Antibiotic consumption in the last 12 months (%)	8 (50)

BMI, body mass index; GERD, gastroesophageal reflux disease; PPI, proton-pump inhibitor.

Antibiotic susceptibility testing confirmed resistance to clarithromycin, metronidazole, and levofloxacin in all 16 isolates. Genotyping revealed mutations in 23S rRNA in 15 cases (93.8%) and mutations in gyrA in 14 (87.5%).

Adverse effects (Table 3) were reported in five patients (31.2%), being mild in four and severe in one. This patient suspended therapy prematurely and was excluded from per-protocol analysis.

Patients' median assessment of the treatment in terms of tolerance and efficacy, according to a visual scale, was 8 (range: 2–10) and 6 (range: 2–9), respectively.

Control UBT was positive in all the 16 patients. Per-protocol and intention-to-treat eradication rates were 0%.

Discussion

H. pylori eradication may fail in up to 40% of cases after second-line regimens. In clinical practice we are frequently confronted with patients for whom two or more treatment attempts were unsuccessful [8]. Our patients had a mean of 2.8 eradication failures before inclusion. After failure of two consecutive treatments including clarithromycin and metronidazole, at least single and frequently double resistance of *H. pylori* is common [31].

Salvage treatments should be determined according to the results of culture and susceptibility tests or, as alternative, to the results of molecular methods allowing detection of resistance to clarithromycin and levofloxacin. However, culture implies general endoscopic risks, is expensive, labor-intensive, not always available on a routine basis, and its sensitivity is not 100% [32]. Molecular methods are also not accessible in all centers and only two drugs can be tested. On the other hand, the choice of drugs for salvage therapy depends on which treatments were formerly used because re-treatment with the same drugs is not recommended except for amoxicillin [31]. In our study, clarithromycin and levofloxacin were already used in 93.7% of patients and nitroimidazoles in 75%. Unfortunately, antibiotic consumption in Portugal is very high. A detailed history of previous infections and antibiotic consumption allowed us to determine that even the patients without prior *H. pylori* eradication attempts had once used macrolides, fluoroquinolones, and/or nitroimidazoles for the treatment of respiratory and/or gynecological infections. So, all 16 patients had past history of clarithromycin, levofloxacin, and nitroimidazoles consumption and *H. pylori* resistance to all of them was expected. Also, as previously reported, there were no cases of resistance to amoxicillin, even after multiple failed treatments using this drug. New therapeutic regimens with clarithromycin, nitroimidazoles, or levofloxacin were not recommended. The only exception could be, eventually, quadruple therapy containing bismuth, PPI, tetracycline, and metronidazole because this regimen can overcome, at least partially, the negative effect of metronidazole resistance [7,8,31].

In countries like Portugal, where bismuth, tetracycline, furazolidone are unavailable and rifabutin use is very restricted, it is almost impossible to delineate a salvage therapy after use of metronidazole/tinidazole, clarithromycin, and levofloxacin. An alternative to tetracycline HCl could be doxycycline that belongs to the same therapeutic group. Heep et al., found no cases of *H. pylori* resistance to doxycycline in isolates from patients in whom one or more therapies had failed, but another work identified a resistance rate of 33.3% [12,33].

Doxycycline, a synthetic antibiotic developed in 1967, theoretically would have some advantages over tetracycline: half-life of 18 hours allowing one or two daily administrations instead of four; better tissue penetration; better absorption with food; predominantly extrarenal excretion; reduced gastric chelation with bismuth if this drug is used concomitantly [11,34,35].

Different treatment regimens for *H. pylori* eradication containing doxycycline were already published. However, the proposed combination of drugs, treatment duration, and final results were very heterogeneous and difficult to compare. Eradication rates on intention-to-treat protocol differed from 36 to 91%, being higher

Table 2 Drugs used in the previous eradication attempts

Drugs	n = 16
Amoxicillin (%)	16 (100)
Clarithromycin (%)	15 (93.7)
Nitroimidazoles (%)	12 (75)
Metronidazole (%)	6 (37.5)
Tinidazole (%)	4 (25)
Metronidazole and tinidazole (%)	2 (12.5)
Levofloxacin (%)	15 (93.7)

Table 3 Reported adverse events

Adverse events	n = 16
Abdominal pain (%)	4 (25)
Nausea (%)	3 (18.8)
Headache (%)	1 (6.3)
Severe diarrhea and vomiting (%)	1 (6.3)

for quadruple regimens [8,11–19]. Treatment with PPI, amoxicillin, and doxycycline, although suboptimal, would allow eradication of *H. pylori* in, at least, one-third of patients. Given all difficulties with rescue regimens in our country, we tried to optimize therapy using pretreatment and high-dose PPI.

Proton-pump inhibitors influence the efficacy of *H. pylori* eradication, and its action depends on the CYP2C19 genotype. Theoretically, standard PPI doses would be insufficient in extensive metabolizers to accomplish enough pH inhibition for efficient antibiotic activity in gastric mucosa, determining lower eradication rates [36]. Westerners, as our patients, are generally rapid metabolizers and might benefit from higher PPI dosages [37]. Graham et al., also observed that the treatment success was ~5% higher when the PPI was given at twice the standard dose, and the last Maastricht IV/Florence consensus established that there is indirect and direct evidence that high-dose PPI can improve the cure rates of *H. pylori* treatment [2,5]. A recent meta-analysis also assumed that increasing PPI dose above the current standards could improve cure rates of *H. pylori* therapy [38]. Because we had no possibility to determine CYP2C19 genotype, we decided that it was acceptable to use high-dose PPI.

Pretreatment with PPI is controversial, and a meta-analysis showed no differences in eradication rates between patients with or without pretreatment [39]. Some older data suggested that pretreatment with a PPI before the administration of *H. pylori* eradication therapy might decrease treatment efficacy, but the meta-analysis and more recent articles do not confirm this information [39–43]. Given the special circumstances of our patients, we decided that such a strategy, albeit a potential confounding factor, could be beneficial.

However, our eradication rate was 0% with this regimen. This was rather unexpectable given the results of previous published series and the in vitro susceptibility to amoxicillin and tetracycline. With the simple combination of high-dose PPI and amoxicillin, we were expecting, at least, a modest rate of eradication, according to the results of dual therapy [41]. Side effects and no compliance were not responsible for such a failure because only one patient experienced severe adverse events and had to suspend treatment. Probably, as some authors already stated, there is an antagonistic effect between these two antibiotics, because doxycycline has a bacteriostatic action and amoxicillin has a bactericidal one. The bacteriostatic effect of doxycycline inhibits ribosomal protein synthesis and may prevent binding of amoxicillin to its bacterial cell wall target [13,16]. Doxycycline in the gastric lumen can also interfere with topical action of amoxicillin and vice versa [13]. On the other side, some authors defend that when we perform susceptibility tests we must use the compound indicated and, for example, we cannot substitute tetracycline HCl by doxycycline [2]. In fact, we did not test susceptibility of *H. pylori* isolates to doxycycline, but other authors defended that metronidazole and tetracycline can be replaced by tinidazole and doxycycline, respectively [27]. Another possibility is that the higher dosage of PPI was ineffective in maintaining pH consistently above 6 and more important than give higher doses two times a day would be to increase the frequency of administration. The type of PPI could also be important. A recent meta-analysis demonstrated that the degree of acid inhibition is not the same for all PPIs. Based on the mean 24-hour gastric pH, the relative potencies of esomeprazole and rabeprazole are higher than the ones for omeprazole and pantoprazole [44]. As previously discussed, instead of the traditional 40 mg b.i.d., we administered 80 mg of pantoprazole b.i.d. Because PPIs have better efficacy in *H. pylori*-infected patients, these dosages were, theoretically, sufficient. Even so, we assume that higher doses of pantoprazole or even new-generation PPIs such as rabeprazole and/or esomeprazole would be acceptable and eventually more effective [45].

We can also speculate that drug dosage or treatment duration was inadequate. Previous studies with doxycycline had durations from 7 to 14 days, and with few exceptions, this antibiotic was administered two times a day. In our study, we tried to optimize compliance and we decided that all drugs would be administered twice daily during 10 days, with PPI pretreatment for 3 days (total duration of treatment – 13 days). Doxycycline has a long half-life, and two daily administrations are enough, but for PPI and amoxicillin four daily administrations would probably be better. However, albeit we applied a simplified therapeutic regimen, with high compliance rates, no *H. pylori* eradication was achieved in our study. So, it is questionable if it is worthwhile to try a more complicated regimen, with four daily administrations of PPI and amoxicillin and twice a day doxycycline, for 14 days. This therapy would be eventually more effective but with increased pill burden and secondary effects, compromising patient tolerability and adherence.

Besides the problems already discussed, our study has some other limitations: a small number of patients; the use of Etest to determine susceptibility to metronidazole; lack of molecular methods to confirm metronidazole resistance.

The small number of patients is easily explained by our study design. We consecutively enrolled 16

patients, but with the first six eradication failures, we prematurely finished inclusion.

Metronidazole susceptibility testing by Etest can be overestimated by 10–20% in comparison with determination by the agar dilution method, and both methods are not sufficiently reliable to determine susceptibility of *H. pylori* to this antibiotic because they lack reproducibility [24]. We tried to overcome this problem by using a control strain and repeating the test by another microbiologist. We must also remember that all patients had already taken nitroimidazoles and this can explain the presence of *H. pylori* resistance to nitroimidazoles.

Finally, the molecular methods confirmed the presence of 23S rRNA and gyrA mutations in 93.7 and 87.5% of *H. pylori* isolates, respectively. These results are perfectly plausible because the methodology used detected the most common mutations. The molecular mechanisms of metronidazole resistance in *H. pylori* are more complex and there are no easily implementable methods to detect such modifications [9,46].

The main question now is what alternative can we offer to our patients as we are dealing with triple-resistant strains. In ideal conditions there would be five options: a triple therapy with rifabutin; quadruple therapy with tetracycline or amoxicillin and furazolidone; quadruple treatment regimen with tetracycline and metronidazole; concomitant or hybrid therapy; and dual therapy with high doses of PPI and amoxicillin. Given the limitations for our country, only the last two can be considered.

Hybrid and concomitant therapies, including PPI, amoxicillin, clarithromycin, and metronidazole, are recently proposed therapies. Apparently, these regimens, due to prolonged duration of amoxicillin administration, have high efficacy in the treatment of *H. pylori* strains harboring dual resistance to clarithromycin and metronidazole. However, further studies in populations with different levels of prevalence of clarithromycin and metronidazole resistance are needed to assess the efficacy of the new regimen [3,47].

The old-fashioned but recently revived dual therapy with PPI and amoxicillin for 14 days could be a good option because it is easily available and, in patients with previous failed treatments, its efficacy is comparable to the one of rifabutin-based therapy [48]. Use of a new-generation PPI and 4 daily administrations of this drug and amoxicillin seem to be of major importance to achieve higher eradication rates [37,45,49]. A trial performed in another South European country revealed disappointing results with dual therapy [50]. In this study, both drugs were administered two and not four times a day and that probably could have influenced the final results.

Conclusion

A triple therapy including PPI in high doses, amoxicillin, and doxycycline, although well tolerated, is useless for the eradication of multidrug-resistant *H. pylori*.

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