ORIGINAL ARTICLE 10.1111/1469-0691.12701

Helicobacter pylori antimicrobial resistance rates in the central region of Portugal

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Abstract

Helicobacter pylori resistance to antimicrobial agents is steadily increasing. It is extremely important to be aware of the local prevalence of antibiotic resistance so as to adjust treatment strategies. During this single-centre, prospective study, we aimed to determine primary and secondary resistance rates of *H. pylori* to antibiotics as well as host and bacterial factors associated with this problem. Overall, 180 patients (131 female; mean age 43.4 ± 13.5 years; primary resistance 103; secondary resistance 77) with positive ^{13}C-urea breath test were submitted to upper endoscopy with gastric biopsies. *Helicobacter pylori* was cultured and antimicrobial susceptibility was determined by Etest and molecular methods. Clinical and microbiological characteristics associated with resistance were evaluated by logistic regression analysis. Among the 180 isolates 50% were resistant to clarithromycin (primary 21.4%; secondary 88.3%), 34.4% to metronidazole (primary 29.1%; secondary 41.6%), 33.9% to levofloxacin (primary 26.2%; secondary 44.2%), 0.6% to tetracycline and 0.6% to amoxicillin. Being female was an independent predictor of resistance to clarithromycin and metronidazole. Previous, failed, eradication treatments were also associated with a decrease in susceptibility to clarithromycin. History of frequent infections, first-degree relatives with gastric carcinoma and low education levels determined increased resistance to levofloxacin. Mutations in the *23S rRNA* and *gyrA* genes were frequently found in isolates with resistance to clarithromycin and levofloxacin, respectively. This study revealed that resistance rates to clarithromycin, metronidazole and levofloxacin are very high and may compromise *H. pylori* eradication with first-line and second-line empiric triple treatments in Portugal.

Keywords: Antibiotics, *Helicobacter pylori*, multidrug resistant, mutations *23S rRNA*, mutations *gyrA*, primary resistance, secondary resistance

Original Submission: 5 April 2014; **Revised Submission:** 25 May 2014; **Accepted:** 27 May 2014

Editor: F. Megraud

Article published online: 31 May 2014

Clin Microbiol Infect

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Introduction

Helicobacter pylori is responsible for multiple gastric pathologies [1]. The universally accepted empiric triple treatment for *H. pylori* comprises proton-pump inhibitors (PPI), clarithromycin and amoxicillin or metronidazole [1]. However, the efficacy of the 'legacy' triple therapy has decreased in the last decades and is now inferior to 80% in many countries [1,2]. There are many reasons for therapeutic failure including poor compliance, inadequate dose/duration of therapy, rapid metabolism of PPI, ineffective penetration of antibiotics into the gastric mucosa, antibiotic inactivation by low gastric pH and, most importantly, *H. pylori* resistance to antimicrobial agents [3,4]. Excessive and indiscriminate use of antibiotics is leading to decreased susceptibility of *H. pylori* to such drugs [5].

Resistance of *H. pylori* to antibiotics is common in clinical practice but we must distinguish primary from secondary resistance. Exposure of *H. pylori* to antibiotics during eradication attempts can select bacterial resistance. This occurs

mainly with clarithromycin and levofloxacin but also with nitroimidazoles. Reports about primary resistance are common but data after therapeutic failure is scarcer because patients previously exposed to anti-*H. pylori* treatment are frequently excluded from susceptibility studies [6,7].

Prevalence of *H. pylori* infection in Portugal is 84.2%, 66.2% and 31.6% for adults, teenagers and children, respectively [8–10]. In 2012 the estimated age-standardized incidence and mortality rates for stomach cancer in Portugal were still some of the highest in Europe [11]. For this reason, *H. pylori* will continue to represent a major healthcare problem in this south European country. The European multicentre studies of *in vitro* antimicrobial resistance revealed that in Portugal primary *H. pylori* resistance rates to clarithromycin, levofloxacin and metronidazole were 31.5%, 26.3% and 33.3%, respectively [5,7]. However, there is little information concerning secondary resistance rates and to our knowledge there are no data about this problem in the central region of the country.

The focus of the present study was to evaluate primary and secondary resistance rates of *H. pylori* to antimicrobials in this region of Portugal. As a secondary objective, we intended to establish potential host and bacterial factors associated with resistance to each one of the tested antibiotics.

Patients and Methods

Patients

In this single-centre study patients with dyspepsia, iron-deficient anaemia, need for chronic therapy with PPI and/or first-degree relatives with gastric carcinoma were prospectively considered for inclusion, from September 2009 to October 2013. All of them had a positive ^{13}C-urea breath test. Exclusion criteria were: age <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; use of antibiotics in the last 4 weeks or PPI in the last 2 weeks.

Patients were divided into two groups: Group I—no previous *H. pylori* eradication treatment (primary resistance); Group II—previous, failed *H. pylori* therapy (secondary resistance).

Study design

All patients were submitted to upper endoscopy with biopsies in the antrum and corpus that were immediately placed in independent containers of adequate transport media—Portagerm pylori (bioMérieux Portugal, Linda-A-Velha, Portugal)—at 4°C, and sent to the microbiology laboratory. Urease test and Gram staining of a smear prepared from the biopsy specimen confirmed the presence of *H. pylori*. After manual grinding with disposable material the samples were distributed directly in agar pylori (bioMerieux Portugal, Linda-A-Velha, Portugal). Cultures were incubated for a minimum of 72 h and a maximum of 10 days at 37°C under microaerobic conditions, produced with H_2-CO_2-generating packs (GENbag, bioMérieux Portugal, Linda-A-Velha, Portugal). The *H. pylori* isolates were identified by colony morphology, characteristic spiral morphology on Gram staining, and positive catalase, urease and oxidase tests. Antrum and corpus samples were processed separately.

The MIC for amoxicillin, clarithromycin, metronidazole, levofloxacin and tetracycline were determined by Etest (bioMérieux Portugal) and expressed in mg/L. To minimize variations in the results, *H. pylori* ATCC 43504 was used for quality control of the susceptibility assay and a different microbiologist, blinded to previous results, repeated all tests. Strains were considered resistant to amoxicillin, clarithromycin, metronidazole, levofloxacin and tetracycline at MIC >0.5, >1, >8, >1, and >1 mg/L, respectively. MIC values were established according to the data available in 2009, including the CLSI breakpoints [3,12–15]. A strain was considered multidrug-resistant if it had resistance to two or more antibiotics.

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* (A2143G/A2142G, A2142C), the quinolone resistance-determining region (QRDR) of the *gyrA* and the *16S rRNA* genes were detected by real-time PCR using a LightCycler device, as previously described [13,16,17].

The *cagA*, *vacA*, *iceA* and *babA2* genotypes were determined with real-time PCR by using specific primers selected from previously published works [18–20].

Statistics

Categorical variables were presented with their relative and absolute values and quantitative ones were expressed as mean ± standard deviation or median + range. For statistical analysis, Student's *t*-test, Mann–Whitney and Fisher's exact test were used. Significant variables were subsequently included in a binary logistic regression analysis to determine independent risk factors for resistance to each antibiotic. The statistical software package SPSS 20.0 for Windows (SPSS, Chicago, IL, USA) was used.

Ethical considerations

The study was approved by the ethics committee of our Hospital and the Faculty of Medicine, and was performed in