Antimicrobial susceptibility of Helicobacter pylori to six antibiotics currently used in Spain

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Background: Antibiotic resistance is directly related to the loss of efficacy of currently accepted Helicobacter pylori therapies. Knowledge of the antibiotic susceptibility in a local area can contribute to the design of specific ‘à la carte’ treatments. The aim of this study was to analyse the susceptibility of H. pylori isolates to six conventional antibiotics currently used in a northern region of Spain.

Methods: Seventy-one isolates were obtained from gastric biopsies of 76 consecutive adult patients suffering from peptic ulcer disease, dyspepsia or familial gastric cancer and known to be infected with H. pylori by conventional methods. Susceptibility testing was performed for amoxicillin, ciprofloxacin, levofloxacin, clarithromycin, metronidazole and tetracycline using the Etest method.

Results: The prevalence rates of resistance were as follows: amoxicillin, 1.4% [95% confidence interval (CI) 0.0–7.6]; clarithromycin, 14.7% (95% CI 7.3–25.4); ciprofloxacin, 14.3% (95% CI 7.1–24.7); levofloxacin, 14.5% (95% CI 7.2–25.0); metronidazole, 45.1% (95% CI 33.2–57.3); and tetracycline, 0% (95% CI 0.0–5.1).

Conclusions: Our study confirms an increasing rate of resistance to levofloxacin that equals that of clarithromycin in our healthcare area. This fact may reflect a wide and indiscriminate use of the former antibiotic and could account for a loss of clinical effectiveness of levofloxacin-containing regimens. Moreover, clarithromycin resistance rates remain stable, which could allow us to maintain its use in our area.

Keywords: antibiotic resistance, dyspepsia, eradication, peptic ulcer disease, therapeutic regimens

Introduction

Helicobacter pylori infects the gastric mucosa and represents the main cause of gastritis, peptic ulcer disease and gastric cancer. It has been demonstrated that eradication of H. pylori improves the clinical outcome of patients with duodenal ulcer, prevents recurrence and decreases the risk of gastric cancer in infected patients.1,2 Nowadays, the European and American guidelines on the treatment of H. pylori infection recommend as first-line therapy a combination of a proton pump inhibitor (PPI) with two antibiotics; omeprazole and clarithromycin plus amoxicillin or metronidazole being the preferred regimen.3,4 Although the initial eradication success rate for the standard triple therapy was in excess of 90% 10 years ago, therapy failures of up to 30%–40% of cases using this regimen have been more recently reported.5–8 Even though a patient’s lack of compliance, inadequate length of therapy or a high bacterial burden are conditions that may contribute to such a loss of efficacy, antimicrobial resistance is regarded as the leading factor responsible for eradication failure. This issue is of particular relevance with regard to clarithromycin, which can induce a virtually 70% loss of effectiveness when it is part of a PPI/amoxicillin-based triple therapy, depending on in vitro macrolide susceptibility.9 In fact, the most recent Maastricht guidelines on H. pylori infection...
management recommend substituting metronidazole for clarithromycin when resistance to this antibiotic exceeds 15%–20%. Finally, there is wide geographical variation regarding the prevalence of antibiotic resistance. This fact has been recently highlighted in the updated European surveillance of H. pylori resistance to antibiotics, where differences in clarithromycin resistance rates of more than 10% were detected between different regions of Europe, precluding its use in some of them. All of this makes general guidelines related to the use of different antibiotic regimens against H. pylori useless if they do not include the available data about antibiotic susceptibility in local areas. Taking into account the aforementioned considerations, it seems desirable to have regularly updated, reliable information on the prevalence of antibiotic resistance to H. pylori for various countries, regions or healthcare areas. Such information can aid in the establishment, on an individual basis, of the potentially most effective eradicating regimen for H. pylori infection.

The aim of this study was to assess the susceptibility of H. pylori strains isolated from gastric biopsies of patients with gastroduodenal peptic ulcer disease, treatment unresponsive dyspepsia or family history of gastric cancer to six antibiotics commonly used in therapeutic procedures.

**Methods**

**Patients and sample collection**

From February to December 2010, 76 consecutive adult patients who had not been previously eradicated of H. pylori were evaluated at the Gastroenterology Department of the Hospital of Laredo, a community hospital in the north of Spain. These patients referred different upper abdominal complaints, a familial history of gastric cancer and/or a personal history of gastroduodenal peptic ulcer disease. All of them underwent a diagnostic oesophagogastrroduodenoscopy, including collection of biopsies of the gastric mucosa (from both the body and the antrum) for a rapid urease test, histological study and culture.

**Culture preparation and susceptibility testing**

The biopsy specimens of those patients with a positive urease test were homogenized and sowed in selective (Agar Pylori; bioMérieux, Sweden) and non-selective (Columbia III Agar with 5% Sheep Blood; Becton-Dickinson, Germany) culture media and incubated at 37°C for 10–14 days under microaerophilic conditions. The tested drugs were amoxicillin, clarithromycin, ciprofloxacin, levofloxacin, metronidazole and tetracycline. The breakpoints used to classify strains as susceptible or resistant according to the MIC value were as follows: \( \leq 2 \) mg/L = susceptible and \( \geq 2 \) mg/L = resistant for amoxicillin, clarithromycin, ciprofloxacin and levofloxacin; \( \leq 4 \) mg/L = susceptible and \( \geq 8 \) mg/L = resistant for metronidazole; and \( \leq 2 \) mg/L = susceptible and \( \geq 4 \) mg/L = resistant for tetracycline. The breakpoints for amoxicillin, clarithromycin, metronidazole and tetracycline were interpreted according to the BSAC recommendations. Quinolones are not standardized by the BSAC, so we used those recommended by the Société Française de Microbiologie that are in accordance with those suggested by other authors.

**Statistics**

A descriptive analysis was performed using the SPSS v.15.0 software package.

**Ethics**

This study was performed following the current standards of good clinical practice and good laboratory practice and the protocol was approved by the Cantabric Ethical Investigation Committee. An informed consent was obtained from all the patients.

**Results**

Seventy-one H. pylori strains were isolated from 76 consecutive adult patients (31 male and 45 female) who had been included in the study on the basis of a positive rapid urease test. Susceptibilities to the six tested antimicrobials were determined according to the aforementioned Etest method and are summarized in Table 1 (in 4 of 71 isolates antimicrobial susceptibility could not be tested for all antibiotics).

Forty-four of 71 isolates (62%) showed resistance to at least one antibiotic, while in 27 patients all isolates were susceptible to the tested antibiotics. Resistance to only one antibiotic was

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**Table 1. Antimicrobial susceptibility of H. pylori isolates in the north of Spain**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Isolates tested (n)</th>
<th>Isolates with resistance (n)</th>
<th>Resistance, % (95% confidence interval)</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>71</td>
<td>1</td>
<td>1.4 (0.0–7.6)</td>
<td>( \leq 1 )</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>70</td>
<td>10</td>
<td>14.3 (7.1–24.7)</td>
<td>( \leq 1 )</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>69</td>
<td>10</td>
<td>14.5 (7.2–25)</td>
<td>( \leq 1 )</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>68</td>
<td>10</td>
<td>14.7 (7.3–25.4)</td>
<td>( \leq 1 )</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>71</td>
<td>32</td>
<td>45.1 (33.2–57.3)</td>
<td>( \leq 4 )</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>71</td>
<td>0</td>
<td>0.0 (0.0–5.1)</td>
<td>( \leq 2 )</td>
</tr>
</tbody>
</table>

The MIC was determined using the Etest method as recommended by the BSAC. Mueller–Hinton agar supplemented with 5% sheep blood (Becton-Dickinson, Germany) was used as the culture medium. An H. pylori culture suspension with a turbidity equivalent to that of a 3.0 McFarland standard was used to inoculate the plates by confluent swabbing, and Etest strips (bioMérieux, Sweden) were applied onto culture plates. The plates were incubated at 35°C for 3–5 days under microaerophilic conditions. The tested drugs were amoxicillin, clarithromycin, ciprofloxacin, levofloxacin, metronidazole and tetracycline. The breakpoints used to classify strains as susceptible or resistant according to the MIC value were as follows: \( \leq 2 \) mg/L = susceptible and \( \geq 2 \) mg/L = resistant for amoxicillin, clarithromycin, ciprofloxacin and levofloxacin; \( \leq 4 \) mg/L = susceptible and \( \geq 8 \) mg/L = resistant for metronidazole; and \( \leq 2 \) mg/L = susceptible and \( \geq 4 \) mg/L = resistant for tetracycline. The breakpoints for amoxicillin, clarithromycin, metronidazole and tetracycline were interpreted according to the BSAC recommendations. Quinolones are not standardized by the BSAC, so we used those recommended by the Société Française de Microbiologie that are in accordance with those suggested by other authors.
present in the isolates obtained from 31 patients. On the other hand, 13 isolates showed resistance to two or more antibiotics, mainly involving quinolones (five patients). Isolates coming from five patients showed multiresistance, defined as having resistance to three or more antibiotics (in four patients, \textit{H. pylori} strains exhibited resistance to ciprofloxacin, levofloxacin and metronidazole, and in another patient the isolate was also resistant to clarithromycin). Finally, only one patient had an isolate that was simultaneously resistant to levofloxacin and clarithromycin.

**Discussion**

Knowledge of the available data about the \textit{in vitro} antimicrobial susceptibility of \textit{H. pylori} has been requested by several experts in order to increase the therapeutic success rate for \textit{H. pylori} eradication\textsuperscript{11,13}. The rationale for this claim relies on two facts: first, the well-known variability in the prevalence of antibiotic resistance among different countries or even regions or social groups (mainly related to clarithromycin); and second, the special consequences that a wrong antibiotic selection can bear, as has been stated before (e.g. a nearly 70% loss of efficacy depending on susceptibility or resistance to clarithromycin).

In the present study we found a wide spectrum of resistance rates of \textit{H. pylori}, from nearly negligible rates of resistance to tetracycline (0%) and amoxicillin (1.4%) to high rates of resistance to metronidazole (45.1%). Intermediate and virtually identical rates of resistance were found to clarithromycin (14.7%), ciprofloxacin (14.3%) and levofloxacin (14.5%). These results merit some considerations. First, the prevalence rates of \textit{H. pylori} resistance to clarithromycin and amoxicillin are in accordance with those reported in 2001 in Madrid (Spain) within the European multicentre survey of \textit{in vitro} antimicrobial resistance in \textit{H. pylori} (15% and 0%, respectively).\textsuperscript{17} It is important to remark on the minimal variation in resistance patterns found throughout the last decade, particularly with regard to clarithromycin, which could result in a maintained relatively high efficacy of the standard triple therapy against \textit{H. pylori} in our region. These results are in accordance with those reported in a recent European survey, where clarithromycin resistance rates were 17% as a whole.\textsuperscript{16} While countries from the centre, west and south of Europe have experienced a great increase in rates of resistance to clarithromycin (>20%), which jeopardizes its use as part of conventional triple-therapy empirical regimens, northern countries and other exceptions, such as Germany and Spain, maintain low to intermediate rates of resistance. Our group is in the process of conducting a randomized clinical trial on first-line \textit{H. pylori} eradication therapy comparing two triple-therapy regimens (namely omeprazole, amoxicillin and either clarithromycin or levofloxacin), which will help us to clarify the true clinical effectiveness of clarithromycin and levofloxacin in our area. A high rate of eradication with standard first-line therapies has been achieved in other regions on the basis of a high adherence to the treatment, so a combination of a moderate slowly growing rate of resistance to antibiotics and high levels of compliance could account for sustained high eradication rates.\textsuperscript{15,18} Second, the rates of resistance to metronidazole are slightly higher than previously reported in Spain (37.2%) and Europe (33.1%) in 2001, approaching those encountered in Italy (49%), Austria (44.9%) and Greece (44.1%).\textsuperscript{17} Certainly, metronidazole resistance has remained stable in the last decade in Europe as a whole [34.3%; 95% confidence interval (CI) 16.7–50.3].\textsuperscript{10} Although individual figures of each country are awaited and differences between them, as those found for clarithromycin, can be expected to confirm our results. Even though \textit{in vitro} resistance to metronidazole may not accurately reflect \textit{in vivo} resistance, regimens including metronidazole are not a preferable choice in populations with >40% metronidazole resistance.\textsuperscript{12,19} Thus, our data could dissuade gastroenterologists in our region from using this antibiotic in alternative first-line therapeutic regimens against \textit{H. pylori}, particularly in cases of penicillin allergy.

Third, and of great interest in this study, is the notable rate of \textit{H. pylori} resistance to levofloxacin, a quinolone increasingly used as a clarithromycin substitute for either first-line or rescue therapy in different regimens.\textsuperscript{20} In spite of the high eradication rates (~90%) achieved with the combination of PPI, amoxicillin and levofloxacin, there are concerns about an increasing rate of quinolone resistance: 15% in Japan; 16.8% in Belgium; 23.1% in Italy; from 2.8% in 1998 to 11.8% in 2003 in Taiwan; from 3% in 1999 to 15% in 2004 in France; and from 11.2% in 2003 to 22.1% in 2005 in Germany.\textsuperscript{8,16,20–24} These changes in \textit{H. pylori} susceptibility to quinolones could account for a decrease in the success rate of a triple therapy including levofloxacin. In fact, some investigators have linked the slight reduction in overall eradication rates of a levofloxacin-based re-treatment (from 76%–85.7% to 72.7%) to the high prevalence of \textit{in vitro} primary resistance (30.3%), which doubled that found in previous trials.\textsuperscript{20} Although a relatively low rate of \textit{H. pylori} resistance to quinolones (6%) had been previously reported in Spain,\textsuperscript{25} the present study reveals and confirms an increasing rate of levofloxacin resistance in our country, which is similar to what has been reported in other Mediterranean areas. In this way, the aforementioned European study on antibiotic resistance of \textit{H. pylori}\textsuperscript{10} underscores a progressive trend of higher resistance rates to levofloxacin (similar to those encountered in our study), which could discourage the future use of eradication regimens including this quinolone. Finally, resistance to amoxicillin and tetracycline remains negligible.

To sum up, the present work shows stable \textit{in vitro} resistance rates of \textit{H. pylori} to clarithromycin, which could support its use as a part of \textit{H. pylori} eradication regimens in our area. In addition, it also confirms a rapid increase in the \textit{in vitro} resistance rate to levofloxacin in our region, which may discourage its use in eradication regimens, at least as first-line treatment. Taking into account the important variability of prevalence rates of \textit{H. pylori} resistance to different antibiotics (in time and space) and the consequences that this fact can have on therapy success, we encourage regional gastroenterology and microbiology societies to periodically update their data on \textit{in vitro} resistances and make suitable recommendations about the best desirable therapy.

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Searching for appropriate H. pylori eradication antibiotics

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Transparency declarations
None to declare.

References