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Original article

Helicobacter pylori resistance to antibiotics in 2014 in France detected by phenotypic and genotypic methods

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ABSTRACT

A large survey of antimicrobial resistance of *Helicobacter pylori* was performed in France in 2014: 984 patients were enrolled by 75 gastroenterologists all over the country. Among the 783 patients who had never received eradication treatment before, 266 (33.9%) were *H. pylori* positive. The strains showed a high rate of clarithromycin resistance (22.2%), moderate rate of resistance to levofloxacin (15.4%) and high rate of resistance to metronidazole (45.9%). In all, 187 patients had received previous treatment, of which 115 were *H. pylori* positive with very high resistance to clarithromycin (73.9%) and metronidazole (78.3%). None of the patients receiving PYLERA (Bismuth salt-Tetracycline HCl-Metronidazole) proton-pump inhibitor developed resistance to tetracycline. A real-time PCR applied to gastric biopsy specimens detected all the cases that were positive by culture as well as 30 additional cases. A good correlation was found between the clarithromycin resistance detected by phenotypic methods and the associated mutations for clarithromycin resistance, which has continued to increase in the last decade but at a lower rate than previously observed. **A. Ducournau, CMI 2016;22:715**

Introduction

Bacterial resistance to antibiotics is an evolving process that requires periodic surveys. Indeed, for *Helicobacter pylori*, resistance to clarithromycin has been shown to be the main cause of failure of the standard triple therapy used for 20 years but now reaching unacceptable levels of failure, i.e. >15% [1].

The introduction of a new treatment, a bismuth-based quadruple therapy namely PYLERA® (a three-in-one capsule containing bismuth salt, tetracycline and metronidazole)–proton-pump inhibitor (PPI), also raises the question of the possible emergence of tetracycline resistance in *H. pylori*.

This study was carried out in France to evaluate the current rate of *H. pylori* resistance to clarithromycin and to five other antibiotics of potential use to treat *H. pylori* infection, in patients who have or have not previously received eradication treatment, and to look for differences according to the defined regions. Particular attention was paid to resistance to tetracycline in patients who received PYLERA.

Materials and methods

Study population

The aim was to select 100 gastroenterologists distributed in five large regions of metropolitan France (Northeast, Northwest, Southeast, Southwest and Paris area). These gastroenterologists were randomly selected from an exhaustive database of gastroenterologists ($n = 2075$) according to the population distribution.

Inclusion was based on patients aged ≥18 years, scheduled for an endoscopy, exhibiting a pathology potentially associated with *H. pylori* infection, including those who had failed eradication, with signed informed consent. The aim was to include 1000 patients within a year with a maximum of 20 patients per centre.

This study was approved by the ethical committees under No. 13.470 in September 2013.

Methods used

Data were collected on case report forms and concerned socio-demography, reasons for consultation, use of antibiotics in the previous 30 days, history of infection as well as previous

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eradication attempts including the use of PYLERA-PPI, and endoscopy results.

During upper digestive endoscopy, biopsies were obtained from the antrum and body of the stomach and put together in a Portagerm pylori (bioMérieux, Marcy l'Etoile, France).

The Portagerm pylori was sent, maintained at 4°C, in a van to a central facility, the National Reference Centre for Helicobacters and Campylobacters, in Bordeaux, France, and was most often received the following day.

In the laboratory, the biopsies were ground together (antrum and body) using a manual disposable homogenizer (Eppendorf) in 1 mL of broth and part of the suspension was plated without delay on pylori agar (bioMérieux) and Wilkins Chalgren agar (made in-house) supplemented with 10% human blood and the following antibiotics: cefsulodin, trimethoprim, vancomycin and amphotericin B. The plates were incubated at 37°C in a microaerobic atmosphere (5% O_2, 10% CO_2 and 85% N_2) in a special cabinet, and observed for growth after 2 days and every day for 10 days before being discarded if negative. Colonies suggesting *H. pylori* were tested for oxidase, catalase and urease and observed by microscopy [2].

When positive for *H. pylori*, antimicrobial susceptibility testing was performed according to the following method: preparation of a McFarland 3 equivalent standard suspension; inoculation on a Müller–Hinton agar supplemented with 10% sheep blood; placement of Etests for clarithromycin, amoxicillin and metronidazole, and discs for tetracycline, rifamycin and levofloxacin; and incubation for 48 h at 37°C in a microaerobic atmosphere. Discs were used instead of Etests for tetracycline and rifamycin because of the usual low MIC easily detected in this way. EUCAST criteria were used to determine resistance to clarithromycin, amoxicillin and metronidazole (http://www.eucast.org). For the other antibiotics, the following cut-off diameters (mm) were used: tetracycline: resistant <17, susceptible ≥19; rifamycin: resistant <14, susceptible ≥19; and levofloxacin: resistant <17, susceptible ≥20. As no cut-off was recommended for these antibiotics, we performed a study on the distribution of the diameters of 388 strains, which showed a normal distribution confirming the cut-offs chosen for rifampicin and tetracycline. For levofloxacin, MICs were determined by Etest on the strain with intermediate susceptibility. The *H. pylori* strain CCUG 17874 was used for quality control.

In parallel DNA was extracted from the gastric tissue suspension with MagnaPur 96 (Roche Diagnostic, Meylan, France) after proteinase K digestion at 56°C overnight and an in-house real-time PCR developed in the laboratory was used [3]. Sequencing of the target genes was performed in the case of unusual mutation for clarithromycin resistance or for rifamycin resistance and MICs controlled by Etest.

Results

Patient characteristics

In this study, 984 patients were enrolled by 75 gastroenterologists over a 1-year period (February 2014 to February 2015). The median number of patients included per gastroenterologist was 14 (extremes 1–20). Among these patients, 434 (44.1%) were men, and their mean age was 51.5 years ± 15.9. Most of the patients were born in France (718; 73%); the second and third countries of birth were Morocco (54; 5.5%) and Algeria (50; 5%), with the remainder born in 59 different countries worldwide. They were living in 60 different departments out of a total of 96 in metropolitan France. The main symptom warranting endoscopy was epigastric pain in 667 (67.8%). Peptic ulcer was observed at endoscopy in 84 patients (8.5%) and erosions in 203 (20.6%).

Most patients (783; 79.6%) had not received previous *H. pylori* eradication therapy, but 187 (19%) were previously treated, including 57 patients given PYLERA-PPI (30.5%). For 14 patients, it was not known if they had received previous eradication treatment.

Detection of H. pylori by culture and antimicrobial susceptibility testing

Culture performed on antral and fundic biopsies of these 984 patients was positive for *H. pylori* in 386 (39.2%) including 266 of the 783 (33.9%) who had never received previous eradication therapy, 115 of the 187 (61.4%) who received this treatment and five for whom the information was not known.

The results of antimicrobial susceptibility testing are presented in Table 1 indicating a high level of clarithromycin resistance (22.2%) among strains from patients who never received any eradication treatment. We did not find any association with gender, or the country of origin (France versus other countries). However, there was an association with age, the patients aged ≥50 years were more likely to harbour clarithromycin-resistant strains than the younger patients (p 0.02).

In contrast, the proportion of resistant strains was comparatively limited for levofloxacin (15.4%). Metronidazole exhibited a very high level of resistance (>40%). No resistance to tetracycline was detected using the disc diffusion method. The three cases of rifamycin resistance were confirmed by sequencing of the *rpo*B gene. The mutations observed were S526T, D530N (already reported [4,5]) and L547F (detected and confirmed by another group in France; J. Raymond, Paris, personal communication). These MICs were ≥32 mg/L.

Secondary resistance corresponding to the cases of patients who already received *H. pylori* eradication treatment, was much higher for clarithromycin and metronidazole, i.e. 73.9% and 78.3%, respectively.

We did not find statistically significant differences in the prevalence of resistance among the five French regions

Detection of H. pylori and the mutation associated with macrolide resistance by real-time PCR

The real-time PCR applied to gastric biopsies from the 984 patients was positive for 416 patients (42.3%). This number includes the 386 positive by culture plus 30 new cases (3%).

Globally there were 158 cases with mutations compared with 144 with resistance detected by antimicrobial susceptibility testing, i.e. a slightly higher proportion (37.9% versus 37.7%, respectively).

The mutations found were principally A2142/2143G (150); there were only six A2142C mutations and two A2142T mutations confirmed by sequencing. The MICs of these last two strains were 8

Table 1
Prevalence of *Helicobacter pylori* resistance to antibiotics in France in 2014 according to absence of (primary resistance) or previous (secondary resistance) eradication treatment

	Primary resistance ($n = 266$)		Secondary resistance ($n = 115$)	
	No. Resistant	% Resistant (95% CI)	No. Resistant	% Resistant (95% CI)
Clarithromycin	59	22.2 (17.3–27.7)	85	73.9 (64.9–81.7)
Levofloxacin	41	15.4 (11.3–20.3)	17	14.8 (8.9–22.6)
Metronidazole	121	45.9 (39.8–52.1)	90	78.3 (69.6–85.4)
Tetracycline	0	0	0	0
Rifamycin	2	0.7 (0.1–2.7)	1	0.9 (0–4.7)
Amoxicillin	2	0.7 (0.1–2.0)	0	0