

## Primary antibiotic resistance of *Helicobacter pylori* strains isolated from Portuguese children: a prospective multicentre study over a 10 year period

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**Objectives:** The aim of this study was to prospectively assess the pattern of evolution of primary resistance to antibiotics in *Helicobacter pylori* strains isolated from Portuguese children over a 10 year period (2000–09).

**Methods:** A total of 1115 *H. pylori* strains were tested for antibiotic susceptibility to clarithromycin, metronidazole, amoxicillin, ciprofloxacin and tetracycline.

**Results:** *H. pylori* strains were isolated from children and adolescents [ages 4 months–18 years (mean age 10.17 ± 4.03 years)], comprising 562 (50.4%) boys and 553 (49.6%) girls. Overall, the primary resistance rate was 34.7% to clarithromycin, 13.9% to metronidazole and 4.6% to ciprofloxacin, while 6.9% were resistant to two of these antibiotics simultaneously. Resistance to amoxicillin and to tetracycline was not detected. In general, the resistance rate was not associated with gender or the children's age. European ethnicity, when compared with an African background, was associated with clarithromycin resistance [ $P=0.002$ ; odds ratio (OR)=0.30; 95% confidence interval (CI) 0.14–0.66], while the inverse situation was observed for metronidazole ( $P<0.001$ ; OR=3.50; 95% CI 1.90–6.45). No significant temporal trend was noticed for resistance to clarithromycin and metronidazole, whereas ciprofloxacin and double-resistance rates have significantly increased over time ( $P=0.004$  and  $P=0.05$ , respectively).

**Conclusions:** The primary resistance rate of *H. pylori* strains isolated from Portuguese children to the commonly used anti-*H. pylori* antibiotics used is high. Additionally, the increasing trend of ciprofloxacin-resistant and double-resistant strains may compromise *H. pylori* eradication in a high-prevalence population.

**Keywords:** *H. pylori*, temporal trend, Portugal

### Introduction

*Helicobacter pylori* colonizes the human gastric mucosa of more than 50% of the world's population, with infection always eliciting an acute immune response that fails, however, in achieving spontaneous eradication.<sup>1</sup> The lifelong persistence of this infection may result in the development of non-ulcer dyspepsia (NUD), which may further progress to severe gastric diseases, such as peptic ulcer disease (PUD) and two forms of gastric cancer [adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma].<sup>2</sup>

The most common first-line regimen for the eradication of *H. pylori* consists of a combination of clarithromycin or metronidazole, with amoxicillin, plus a proton pump inhibitor (PPI). Proposed rescue treatment regimens include the use of fluoroquinolones, tetracyclines or rifamycin-based therapies. Considering both the large percentage of the infected population and the high and increasing rates of antibiotic resistance reported worldwide, the issue of treatment is thus currently unsolved and particularly problematic.<sup>3</sup>

The actual prevalence of *H. pylori* antibiotic resistance among children in Portugal is not known. The present study aimed to

prospectively assess the pattern of evolution of primary resistance to antibiotics in *H. pylori* strains isolated from Portuguese children over a 10 year period (2000–09), as well as identify the possible associated factors.

## Methods

### Patients and *H. pylori* strains

From January 2000 to December 2009, a total of 1115 *H. pylori* strains were prospectively isolated from Portuguese children and adolescents attending the three Paediatric Gastroenterology Units in the Lisbon area for severe and/or recurrent upper gastrointestinal symptoms suggestive of organic disease and considered sufficiently relevant to justify an upper endoscopy and antibiotic treatment. Demographic and clinical data were collected retrospectively. None of the patients had been treated for *H. pylori* infection before endoscopy. Clinical strains were cultured from a pool of colonies from antral biopsy specimens.

### Antibiotic susceptibility testing

Susceptibility testing was performed using Etest (bioMérieux, Marcy l'Étoile, France) for clarithromycin, metronidazole and amoxicillin, and by disc diffusion (Oxoid, Hampshire, UK) for ciprofloxacin and tetracycline. The MIC breakpoints used were  $\geq 1$  mg/L for clarithromycin,  $> 8$  mg/L for metronidazole and  $> 0.5$  mg/L for amoxicillin, according to previous references.<sup>4</sup> By disc diffusion, strains were classified as resistant if growth inhibition zones were  $< 20$  mm for ciprofloxacin (5  $\mu$ g) and  $< 17$  mm for tetracycline (30  $\mu$ g) [Comité de l'Antibiogramme de la Société Française de Microbiologie, Recommendations 2010 (Edition de Janvier 2010), <http://www.sfm.asso.fr/>].

### Statistical analysis

Statistical analysis was performed with the statistical package PASW Statistics version 18.01 (IBM SPSS Statistics, release 18.0, July 2009). Pearson's  $\chi^2$  test and 95% confidence intervals (CIs) were used. Identification of variables associated with *H. pylori* antibiotic resistance was performed by univariate logistic regression and confirmed by multiple logistic regression analysis. The level of significance was set at 5%.

## Results

### Population characteristics

Within a 10 year observation period, 1115 *H. pylori* strains were isolated from children and adolescents, ages 4 months–18 years (mean age  $10.17 \pm 4.03$  years), comprising 562 (50.4%) boys and 553 (49.6%) girls. Children were stratified into three age groups: 0–5 years (163, 14.6%); 6–10 years (430, 38.6%); and 11–18 years (522, 46.8%). Information on ethnicity was available for 615 (55.2%) children, with the following distribution: 554 (90.1%) European [mostly Portuguese (98%)], 53 (8.6%) African and 8 (1.3%) Asian or Caucasian/European. According to clinical outcome, most of the children (96%,  $n=1069$ ) presented with NUD (comprising severe recurrent abdominal pain, reflux-like dyspepsia, vomiting, coeliac disease and unexplained refractory sideropenic anaemia) and 4.0% ( $n=44$ ) had PUD. This latter condition was significantly more common in older children [5.4% versus 2.8%;  $P=0.021$ ; odds ratio (OR)=2.12; 95% CI 1.13–3.99] and also in boys (63.6% versus 36.4%;  $P=0.025$ ; OR=2.04; 95% CI 1.09–3.82).

### Antibiotic resistance

Overall, the primary resistance rate of *H. pylori* was 34.7% ( $n=387$ ) to clarithromycin (median MIC 0.016 mg/L, range 0.016–256), 13.9% ( $n=155$ ) to metronidazole (median MIC 0.25 mg/L, range 0.016–256) and 4.6% ( $n=51$ ) to ciprofloxacin. Simultaneous resistance to two of these antibiotics occurred in 6.9% ( $n=76$ ) of the isolates, of which 72.4% ( $n=55$ ) were resistant to both clarithromycin and metronidazole, 23.7% ( $n=18$ ) to both clarithromycin and ciprofloxacin, and 3.9% ( $n=3$ ) to metronidazole and ciprofloxacin. One strain was simultaneously resistant to these three antibiotics. Resistance to amoxicillin (median MIC 0.016 mg/L, range 0.016–0.5) and tetracycline was not found.

No overall significant trend for resistance to clarithromycin or metronidazole was noticed. Indeed, considering three time periods, 2000–02 ( $n=191$ ), 2003–05 ( $n=320$ ) and 2006–09 ( $n=604$ ), a stationary trend could be observed for both these antibiotics (Table 1), although between 2000–02 and 2003–05, a significant decrease in the metronidazole resistance rate was observed ( $P=0.037$ ) (Table 1). The clarithromycin and metronidazole MIC values were also stable throughout the time period studied (data not shown).

In contrast, the resistance rate to ciprofloxacin has been significantly rising over time, particularly during the period 2006–09 ( $P=0.004$ ), and the same scenario has been observed for the double-resistant strains ( $P=0.050$ ) (Figure 1).

The univariate analysis showed that, overall, there was no association of resistance to clarithromycin, metronidazole or ciprofloxacin with children's gender or disease outcome (Table 1), except that metronidazole resistance was more common in older children (group aged 11–18 years), particularly when comparing with the youngest children (13.7% versus 9.9%, respectively;  $P=0.051$ ; OR=1.76; 95% CI 1.00–3.10). Resistance to clarithromycin was associated with European ethnicity, when compared with an African background (37.0% versus 15.1%, respectively;  $P=0.002$ ; OR=0.30; 95% CI 0.14–0.66), while, inversely, resistance to metronidazole was more common among African children (35.8% versus 13.8%, respectively;  $P<0.001$ ; OR=3.50; 95% CI 1.90–6.45). No significantly related factors were related to ciprofloxacin and double resistance (Table 1). All the associations found were confirmed by multiple logistic regression analysis (data not shown).

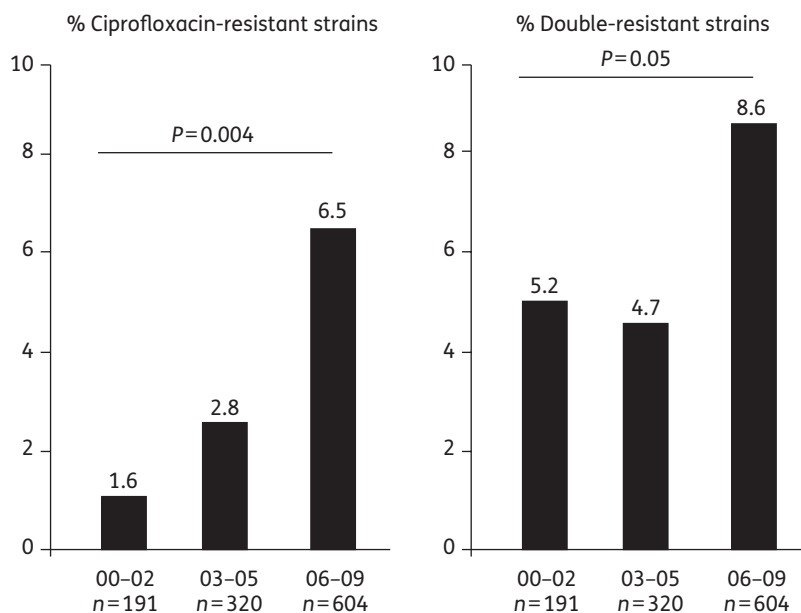
## Discussion

The present study, concerning primary resistance to antibiotics of *H. pylori* strains isolated between 2000 and 2009 from 1115 Portuguese children, shows that the overall resistance rate remains high, in particular to clarithromycin (34.7%). These data are corroborated by a recent European multicentre study on antibiotic resistance of *H. pylori* strains showing that children from south Europe presented a significant risk for primary clarithromycin resistance, which is likely a consequence of a recognized overuse in the past and the persistent use nowadays of macrolides for the treatment of upper respiratory tract infections in those countries.<sup>4</sup> In our study, the only factor associated with clarithromycin resistance was European ethnicity, compared with an African background, as shown in previous studies.<sup>4–6</sup> The high *H. pylori* clarithromycin primary resistance recently reported in

**Table 1.** Univariate analysis of factors associated with *H. pylori* primary resistance to clarithromycin, metronidazole and ciprofloxacin among paediatric patients

Variable	Description	Resistance to clarithromycin [% resistant, OR (95% CI)]	<i>P</i> <sup>a</sup>	Resistance to metronidazole [% resistant, OR (95% CI)]	<i>P</i> <sup>a</sup>	Resistance to ciprofloxacin [% resistant, OR (95% CI)]	<i>P</i> <sup>a</sup>
Gender, <i>n</i> =1115	male	33.6%, 1 (ref)	0.451	12.6%, 1 (ref)	0.863	4.5%, 1 (ref)	0.887
	female	35.8%, 1.10 (0.86–1.41)	0.446	14.2%, 1.04 (0.74–1.46)	0.836	4.7%, 1.06 (0.60–1.85)	0.850
Age (years), <i>n</i> =1115	<6	34.8%, 1 (ref)	0.731	9.9%, 1 (ref)	0.055	3.8%, 1 (ref)	0.831
	6–10	33.3%, 0.93 (0.64–1.37)	0.727	12.0%, 1.22 (0.68–2.24)	0.484	5.0%, 1.33 (0.53–3.36)	0.544
	11–18	35.7%, 1.04 (0.72–1.51)	0.829	13.7%, 1.76 (1.00–3.10)	0.051	4.7%, 1.24 (0.50–3.10)	0.639
Ethnic group, <i>n</i> =615	European	37.0%, 1 (ref)	<b>0.006</b>	13.8%, 1 (ref)	<b>&lt;10<sup>-3</sup></b>	4.7%, 1 (ref)	0.358
	African	15.1%, 0.30 (0.14–0.66)	<b>0.002</b>	35.8%, 3.50 (1.90–6.45)	<b>&lt;10<sup>-3</sup></b>	1.9%, 0.39 (0.05–2.91)	0.357
	other	37.5%, 1.02 (0.24–4.32)	0.977	12.5%, 0.90 (0.11–7.37)	0.918	12.5%, 2.88 (0.34–24.28)	0.331
Gastric disease, <i>n</i> =1113	PUD	22.7%, 1 (ref)	0.106	18.2%, 1 (ref)	0.376	4.5%, 1 (ref)	1.000
	NUD	35.2%, 0.54 (0.27–1.11)	0.094	13.7%, 1.40 (0.64–3.07)	0.401	4.6%, 0.98 (0.23–4.18)	0.982
Time period, <i>n</i> =1115	2000–02	33.5%, 1 (ref)	0.211	16.8%, 1 (ref)	0.074	1.6%, 1 (ref)	<b>0.004</b>
	2003–05	31.3%, 0.90 (0.62–1.32)	0.597	10.3%, 0.57 (0.34–0.97)	<b>0.037</b>	2.8%, 1.81 (0.48–6.77)	0.378
	2006–09	36.9%, 1.16 (0.82–1.64)	0.392	15.0%, 0.87 (0.56–1.36)	0.547	6.5%, 4.35 (1.33–14.24)	<b>0.015</b>

ref, reference category.

<sup>a</sup>*P* value obtained by univariate logistic model.Statistically significant *P* values (<0.05) are highlighted in bold.**Figure 1.** Temporal trend for *H. pylori* resistance to ciprofloxacin and to two of the antibiotics simultaneously (clarithromycin, metronidazole or ciprofloxacin), considering three time periods: 2000–02 (00–02, *n*=191), 2003–05 (03–05, *n*=320) and 2006–09 (06–09, *n*=604).

children from several European countries, as well as from other parts of the developed world, emphasizes that this is a common problem in these populations.<sup>3–6</sup>

The metronidazole primary resistant rate observed in our study (13.9%) was about 2-fold lower than the average rate reported for European children.<sup>4–7</sup> This discrepancy may be

only partially explained by the lower percentage of children with African ethnicity (8.6%) than that reported in other studies. This result also contrasts with the high primary resistance seen among Portuguese adults (32.3%),<sup>8</sup> and likely reflects differences in metronidazole consumption according to the patient's age. Indeed, the lack of a metronidazole paediatric formulation in Portugal may discourage parents to treat their children, resulting in less exposure to this antibiotic.

The high rate of metronidazole resistance detected in our study among African children (35.8%) is in agreement with other reports and consistent with its extensive use for treating parasitic diseases in tropical countries.<sup>4</sup> Metronidazole resistance was also associated with older age, which has not been commonly reported. Indeed, association with age has been described for clarithromycin, but in the opposite sense, i.e. associated with younger age,<sup>4</sup> although in our study, as well as in other studies, this correlation was not found.<sup>5,6</sup>

Data on *H. pylori* quinolone resistance in children is scarce. Nevertheless, the global primary resistance rate to ciprofloxacin observed in our study (4.6%) was higher than that reported in children from Spain (ciprofloxacin, 2/66, 3.6%) or Austria (levofloxacin, 0%).<sup>6,7</sup> Moreover, it has been increasing at an alarming rate over time, reaching almost 7% in the last period studied (2006–09). These results are consistent with data on quinolone consumption among 25 European countries, showing that southern countries presented the highest outpatient use of quinolones, with Portugal occupying the first place.<sup>9</sup>

In conclusion, this is the first *H. pylori* antibiotic resistance multicentre study ever conducted in Portugal, comprising a large number of infected children. We emphasize the findings of high primary resistance rates to the antibiotics most commonly used in the first-line *H. pylori* eradication treatment schemas, with a negative clinical impact in the management of this infection. Furthermore, the increasing detection rate of ciprofloxacin and double-resistant strains over time warrants specific attention and continued surveillance, as it may compromise the efficacy of second-line schemas in the paediatric age group, representing a serious public health problem in populations with a high prevalence of *H. pylori* infection, such as the Portuguese population.<sup>10</sup>

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## Transparency declarations

None to declare.

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