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Prevalence of primary clarithromycin resistance in *Helicobacter pylori* strains over a 15 year period in Italy

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Objectives: The frequency of primary clarithromycin resistance in *Helicobacter pylori* strains is increasing worldwide, and its presence significantly reduces the treatment efficacy of infection. This study aimed to evaluate whether the progression of the prevalence of clarithromycin resistance over a 15 year period has increased and whether a possible change in the distribution of the three most frequent point mutations, which account for the large majority of clarithromycin resistance cases, has taken place.

Methods: Antral biopsies of consecutive *H. pylori*-positive patients observed between January 1989 and December 1990 and between January 2004 and December 2005 were retrieved. A TaqMan real-time PCR was performed in all cases to assess point mutations involved.

Results: Primary clarithromycin resistance was assessed for 147 patients observed in the first period 1989–90 and 178 cases observed in the second period 2004–05. The overall frequency of clarithromycin resistance increased from 10.2% (15 patients) to 21.3% (38 patients) during the study period ($P = 0.01$). The increase was more evident in females [4 out of 55 patients (7.2%) versus 24 out of 103 patients (23.3%); $P = 0.01$] and in non-ulcer dyspepsia patients [13 out of 106 patients (12.2%) versus 37 out of 140 (26.4%) patients; $P = 0.009$]. A2143G was the most frequent point mutation observed in both study periods, and its prevalence rate remained unchanged [11 out of 15 (73.3%) patients versus 27 out of 38 (71%) patients; $P = 1$].

Conclusions: A 2-fold increase in primary clarithromycin resistance in *H. pylori* strains occurred during the last 15 years in Italy. A2143G remains the most prevalent point mutation involved, thus suggesting that new therapeutic strategies are needed.

Keywords: molecular identification, mechanisms of resistance, resistance epidemiology, antibiotic resistance, macrolides

Introduction

Clarithromycin is recognized as the key antibiotic for *Helicobacter pylori* treatment, because of its powerful bactericidal effect *in vitro* compared with the other available molecules.[1] Unfortunately, primary clarithromycin resistance is increasing worldwide, and it has been regarded as the main factor reducing the efficacy of eradication therapy.[2]

In the last decade, novel, culture-free, PCR-based clarithromycin resistance assays have been pioneered allowing the detection of resistant strains with a very high accuracy (98%), even on archival paraffin-embedded biopsy specimens.[3] Among several different single-base mutations, this novel tool is able to identify the three point mutations (A2143G, A2142G and A2142C) in the peptidyltransferase region encoded in domain V of the *H. pylori* 23S ribosomal RNA region, which have been

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detected in >90% of clarithromycin-resistant strains.[3] Interestingly, some *in vitro* studies have found that such genetic mutations are associated with different degrees of bacterial resistance to clarithromycin, although data are conflicting.[3–5] Moreover, we have recently observed that only the A2143G point mutation remarkably reduces the efficacy of a standard triple therapy, whereas the other two mutations failed to significantly affect the cure rate.[6] Therefore, to survey the prevalence of primary clarithromycin resistance and to monitor the distribution of the related point mutations appears essential for the management of *H. pylori* in clinical practice.

The present study aimed to evaluate the change in primary clarithromycin resistance prevalence over a 15 year period and the eventual modification of the distribution of the three most frequent point mutations involved.

Materials and methods

Patients

Antral biopsy specimens of consecutive *H. pylori*-positive patients observed in our Endoscopic Unit (Foggia, southern Italy) between January 1989 and December 1990 (first period) and between January 2004 and December 2005 (second period) were retrieved. *H. pylori* infection was considered present when bacteria were detected at histology (Giemsa staining), jointly with an active chronic gastritis (haematoxylin/eosin) and rapid urease test. To assess primary clarithromycin resistance prevalence, only patients who underwent a first upper endoscopy were considered, whereas those patients previously treated for *H. pylori* infection were excluded from the study. Such information was obtained from the clinical record of each patient. When such clinical data were unavailable, the patient was not enrolled. Non-ulcer dyspepsia was defined as pain or discomfort centred in the upper abdomen without macroscopic lesions at endoscopy, whereas peptic ulcer was defined as a mucosal lesion ≥ 5 mm in diameter in either gastric or duodenal mucosa. Each group of patients included young (<45 years) and old (>45 years) patients.

Clarithromycin resistance assessment

The A2142C, A2142G and A2143G point mutations in the 23S rRNA involved in *H. pylori* clarithromycin resistance were detected by molecular analysis, using a novel method (TaqMan real-time PCR) for *H. pylori* DNA sequencing on paraffin-embedded samples, as we have reported elsewhere.[6,7] The coefficient of TaqMan real-time PCR assay has been shown to be <2%. In our experience, the variability between duplicates and triplicates within the same run or different runs has been 0–2%.

Statistical analysis

Differences between groups were statistically evaluated using the Student's *t*-test for unpaired data, χ^2 test and Fisher's exact probability test. Differences were considered significant at the 5% probability level. Statistical analysis was performed using Statsoft 6.0 program for Windows 98.

Results

Bacterial clarithromycin resistance was assessed for 147 consecutive *H. pylori*-positive patients observed in the first period and 178 patients observed in the second period. The enrolled patients represented 5.4% and 5.6% of all patients who underwent upper endoscopy in our unit in the first and second period, respectively. Demographic and clinical characteristics of the two groups are provided in Table 1.

Overall, a primary clarithromycin resistance was detected in 15 (10.2%) patients in the first period and in 38 (21.3%) patients in the second period. Such a 2-fold increase in the prevalence of clarithromycin resistance was statistically significant ($P = 0.01$). Purely resistant strains (homogeneously resistant bacterial population) were detected in 11 out of 15 patients (73.3%) in the first period and in 14 out of 38 patients (36.8%) in the second period ($P = 0.03$), whereas a co-infection with resistant and susceptible bacterial strains occurred in the remaining cases.

Over the study period, a 3-fold increase in clarithromycin resistance occurred in females [4/55 (7.2%) versus 24/103 (23.3%) patients; $P = 0.009$], whereas only a trend was observed in males [11/92 (11.9%) versus 14/75 (18.6%) patients; $P = 0.3$]. As far as gastroduodenal disease is concerned, a statistically significant increase was found in non-ulcer dyspepsia patients [13/106 (12.2%) versus 37/140 (26.4%) patients; $P = 0.01$], whereas no difference was observed in those with peptic ulcer [2/41 (4.8%) versus 1/38 (5.2%) patients; $P = 1$]. Primary clarithromycin resistance rate similarly increased in those patients <45 years [6/70 (8.6%) versus 16/85 (18.8%) patients] and in those who were older [9/77 (11.7%) versus 22/93 (23.6%) patients]. As shown in Table 2, A2143G was the most frequently observed point mutation in both study periods, and its prevalence rate remained unchanged [11/15 (73.3%) versus 27/38 (71%) patients; $P = 1.0$].

Discussion

Clarithromycin is the most powerful antibiotic included in the standard triple therapies for *H. pylori* eradication endorsed by both European and US guidelines. However, primary clarithromycin resistance is increasing worldwide, with a prevalence rate of 12.9% (6.1–14.5%) in the US and a prevalence rate of as high as 24% in some European countries.[8] A systematic review of *H. pylori* therapy reported a 53% decrease in eradication rate if clarithromycin resistance is present and a clarithromycin-containing regimen is used.[2] In the present study, we observed a 2-fold increase in primary clarithromycin resistance rate over a

Table 1. Baseline demographic and clinical characteristics of patients

	1989–90 (n = 147)	2004–05 (n = 178)	P value
Age (mean ± SD); years	45.8 ± 11.7	47.7 ± 12.2	NS
Young (<45 years)/ old	70/77	85/93	
Male/female	92/55	75/103	0.0004
NUD/PUD	106/41	140/38	NS

NUD, non-ulcer dyspepsia; PUD, peptic ulcer dyspepsia; NS, not significant.

Table 2. Pattern of different point mutations during the study period

	Mutation A2143G	Mutation A2142G	Mutation A2142C
1989–90 (n = 15)	11 (73.3%)	4 (26.6%)	0 (0.0%)
2004–05 (n = 38)	27 (71.0%)	6 (15.7%)	5 (13.3%)

15 year period, reaching values >20% in the last few years. Such a high resistance rate is in agreement with the results of another Italian study,[9] as well as with those performed in other southern European areas, including France (15%), Portugal (22%), Spain (12.9%), Poland (23.5%), Turkey (18.2%) and Bulgaria (12.4%).[1] On the other hand, such an increase was not observed in northern European countries, including Sweden and the Netherlands.[1] Most likely, this discrepancy depends on macrolide consumption in the general population in the different countries. Macrolide consumption, calculated as defined daily doses/1000 inhabitants per day, was much higher in France (3.5), Italy (3.0), Spain (1.9) and Portugal (1.8) than in the Netherlands (0.79) or in Sweden (0.17).[10]

We found that the prevalence of primary clarithromycin resistance was significantly increased in the last few years in non-ulcer dyspepsia patients, whereas no change had occurred in peptic ulcer patients. Such an observation deserves further confirmation in other studies. However, this phenomenon could be involved in the lower eradication rate, following standard triple therapy achieved in dyspeptic patients compared with that reported in those with peptic ulcer. We additionally observed that the prevalence of clarithromycin resistance was significantly increased over the study period only in female patients. It is quite difficult to interpret such a new finding. Interestingly, previous studies had shown a higher primary metronidazole resistance in female patients compared with male patients, most likely due to the wide use of imidazoles for gynaecological infection.[1]

Although several point mutations have been identified, the large majority of the primary clarithromycin resistance cases depend on the three point mutations (A2142C, A2142G and A2143G), which have been related to different MIC values *in vitro*.[1,3–5] The present study confirms that A2143G is by far the most prevalent mutation, accounting for nearly three out of four cases of clarithromycin-resistant strains. Interestingly, the high prevalence of the A2143G mutation observed in our patients agrees with the results of a previous Italian study and other Western studies. Of note, we recently found that only the A2143G point mutation significantly decreased the success rate, following standard triple therapy, whereas the other two did

not.[6] Therefore, the absolute increase in A2143G mutation prevalence observed over the study period in our area could be clinically relevant, suggesting that new molecules and novel drug combinations are needed.

In the last 5 years, we have proposed a novel 10 day sequential regimen that has been found to achieve an eradication rate consistently >90%.[6] Intriguingly, such a therapy regimen appeared to be significantly more effective than 7 or 10 day standard triple therapies, even in the case of clarithromycin-resistant strains harbouring the A2143G point mutation,[6,7] and its use could be suggested as a valid alternative to the standard therapies.

Transparency declarations

None to declare.

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