

Primary clarithromycin resistance to *Helicobacter pylori*: Is this the main reason for triple therapy failure?

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Abstract

Conventional triple therapies for *Helicobacter pylori* (*H. pylori*) eradication have recently shown a disappointing reduction in effectiveness in many countries. The main reason for failure was found to be bacterial resistance to one of the most commonly used antibiotics, clarithromycin. An additional problem for conventional triple therapy is the high rate of resistance to metronidazole found in Europe, America and Asia. In Italy, in the last 15 years a 2-fold increase in resistance has occurred. A recent study of the whole of Italy included about 20 patients from each region at the first endoscopic diagnosis of *H. pylori* infection. The most surprising result was the patchy distribution of resistance, which was almost absent in two regions (one northern and one southern), although the highest prevalence was found in some regions of the South. In the paediatric

population we found a 25% prevalence of resistance in a sample of *H. pylori* positive children observed between 2002 and 2007, mirroring data obtained in southern European countries. Clarithromycin resistance assessment is currently based on phenotypic detection performed after culture the agar dilution method or E-test, and genotypic methods based on polymerase chain reaction (PCR). In a recent comparative study we found a 71.2% agreement between the two methods. Culture-free techniques are highly accurate in finding even minimal traces of genotypically resistant strains. Moreover, PCR-based tools are accurate in detecting a heteroresistant status, defined as the co-existence of some strains that are susceptible and some resistant to the same antibiotic in an individual patient. Three point mutations, namely *A2143G*, *A2142G* and *A2142C*, are responsible for 90% of cases of primary clarithromycin resistance in *H. pylori* strains isolated in Western countries, although we previously demonstrated that the presence of the *A2143G* mutation, but not *A2142G* or *A2142C*, significantly lowered the *H. pylori* eradication rate. Treatment failure has considerable cost/benefit implications because of "waste" of National Health System and patient resources, in terms of drugs, further diagnostic tests and medical examination expenses. Therefore, in future it would be very useful to be able to test for clarithromycin resistance before starting conventional triple therapy. Hopefully, fast, effective non-invasive tests may soon be devised to determine this condition.

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Key words: *Helicobacter pylori*; Clarithromycin; Genotypic resistance; Phenotypic resistance; Therapy failure cost; Resistance epidemiology

Core tip: Clarithromycin resistance is the main reason for the failure of conventional therapies for *Helicobacter pylori*. We evaluate the scope of the problem,

as reported in literature and especially on the basis of personal data in adult and paediatric populations. Another issue is the detection of resistance using phenotypic and genotypic methods; comparison is made of the limits and advantages of these approaches. Cost/benefit analysis of unsuccessful eradication therapy is performed. Based on these considerations, the best solution in future seems likely to be the detection of resistant strains before starting treatment.

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BACKGROUND

Helicobacter pylori (*H. pylori*) eradication may have clinical implications with a very high impact: healing of peptic ulcer disease, resolution of low-grade lymphoma in about 80% of cases and the prevention of gastric adenocarcinoma^[1,2]. However, some problems have arisen in clinical practice in recent years, namely the decreased eradication rate of conventional therapies and the onset and diffusion of antibiotic resistances worldwide^[3]. Triple therapy, comprising treatment with two antibiotics, amoxicillin and clarithromycin, and a proton pump inhibitor for a week, was recommended as the treatment of choice at several consensus conferences. However, this treatment has recently shown a disappointing reduction in effectiveness in many countries and the main reason for failure has been found to be bacterial resistance to one of the most commonly used antibiotics, clarithromycin^[4].

PREVALENCE OF RESISTANCE TO CLARITHROMYCIN

An assessment of primary clarithromycin resistance made about 10 years ago demonstrated differences between children and adults in Europe. In adults, a marked difference has been observed between Northern countries, where the overall prevalence was about 5%, and Southern countries, where the resistance rate was up to 20%. This difference was not observed in children. Indeed, in all European countries a virtually equal, high prevalence has been reported, ranging from 12.5% to 23.5%. An additional problem for conventional triple therapy is the high rate of resistance to metronidazole^[5,6] found in Europe (from 23.5% in Spain to 40.3% in the United Kingdom), America (from 21.6% in the United States to 76.3% in Mexico) and Asia (from 9%-12% in Japan to 41.9% in Korea).

In Italy, data obtained 10 years ago showed a strong difference between the resistance rates in Northern (2%)

and Central (20%) areas^[7,8]. However, in the same period a study by our group found a median value of 28.7% in two areas in Southern and Central Italy^[9]. In 2007, in a retrospective study performed over a 15-year period in the same geographical area, we found a 10.2% prevalence of primary clarithromycin resistance in 147 patients in the 1990 period, whereas by 2004-2005 the resistance found in 178 patients had increased significantly, to 21.3% or 2-fold higher^[10]. The phenomenon was found to be more evident in females. A possible explanation for this finding may be a wider use of the antibiotic in the course of infections with a greater prevalence in the female sex, such as urinary tract infections^[11,12].

A more recent study (2011) was focused on the whole of Italy, including about 20 patients from each region at the first endoscopic diagnosis of *H. pylori* infection. The most surprising result was the patchy distribution of resistance, which was almost absent in two regions (one Northern and one Southern) although the highest prevalence was found in some regions of the South (20%-25%). Due to this distribution variability, the overall resistance rate was lower than expected (about 10%). Resistances were higher in patients with non-ulcer dyspepsia than in those with peptic ulcer, in agreement with findings in previous investigations^[13].

In the paediatric population we found a 25% prevalence of resistance in a sample of 168 *H. pylori* positive children observed between 2002 and 2007, mirroring other data from European countries, where high values reflect the environmental conditions of southern areas^[14].

METHODS FOR CLARITHROMYCIN RESISTANCE DETECTION

Clarithromycin resistance assessment is currently based on phenotypic detection performed after culture and the agar dilution method or E-test. However, in the past decade, different polymerase chain reaction (PCR) based approaches have been developed as alternative tools^[15]. These techniques allow assessment of mutations in the peptidyltransferase region encoded in domain V of the *H. pylori* 23S ribosomal RNA region that confers clarithromycin resistance^[16,17]. Undeniably, both culture and PCR-based methods have both advantages and limitations^[18]. Bacterial culture allows an overall evaluation of *H. pylori* clarithromycin resistance, regardless of the intrinsic mechanism involved (point mutations, RNA methylations, efflux pumps, *etc.*). Nevertheless, *H. pylori* is a troublesome bacterium and culture may be difficult even in expert hands. Indeed, sensitivity values of culture as low as 55%-73% have been reported in some trials^[19]. By contrast, PCR-based culture-free techniques are highly accurate in finding even minimal traces of genotypically resistant strains. Moreover, PCR-based tools are accurate in detecting a heteroresistant status, defined as the co-existence of some strains that are susceptible and some resistant to the same antibiotic in an individual patient. These techniques may be used even on paraffin-embed-

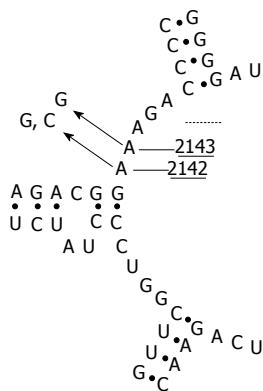


Figure 1 Secondary structure model of the peptidyltransferase region in domain V of the *Helicobacter pylori* 23S rRNA. The site of the A2142C, A2142G and A2143G mutations is underlined.

ded tissue^[20]. Nevertheless, these approaches are unable to detect clarithromycin resistance when it is based on uncommon genetic mechanisms (deletions, RNA methylations, *etc.*). Although several mutations have been detected, it has been found that three point mutations, namely A2143G, A2142G and A2142C (Figure 1), are responsible for 90% of cases of primary clarithromycin resistance in *H. pylori* strains isolated in Western countries^[21,22]. Some studies showed that these point mutations are associated with different MIC values for clarithromycin resistance assessed by culture *in vitro*^[23], suggesting that they might have a different impact on the therapeutic outcome. Indeed, we previously found that the presence of the A2143G mutation, but not A2142G or A2142C, significantly lowered the *H. pylori* eradication rate^[24,25].

In a recent comparative study we found that the prevalence of clarithromycin phenotypic resistance was significantly lower than that of genotypic resistance (18.4% *vs* 37.6%, $P < 0.001$). An agreement between the two methods was present in 71.2% of cases. A significant difference in the eradication rate was seen between clarithromycin-susceptible and resistant strains, when assessed with either the *E* test (92.4% *vs* 55.5%, $P < 0.001$) or a PCR-based method (94.5% *vs* 70.9%, $P < 0.001$). Notably, the eradication rate showed the lowest value (30.7%) when the phenotypic bacterial resistance was genetically linked to the A2143G point mutation. The conclusion of this study was that there is a disagreement between the two methods; clarithromycin resistance markedly reduces *H. pylori* eradication only when it is linked to a specific point mutation^[26].

On these grounds, it has been suggested that MIC values for clarithromycin resistance should be dropped to 0.5 in order to improve the efficacy of phenotypic detection^[27].

COST/BENEFIT IMPLICATIONS IN ITALY

Conventional 7-d triple therapy failure leads to a “waste” of about 27 € in each case of resistance to clarithromycin. The sum is doubled when the therapy duration is prolonged to 10-14 d, as suggested by some reports^[28]. If

sequential therapy is used, the cost of treatment is about 40 € but the therapeutic gain is unsatisfactory.

In addition to the net cost of drugs, the cost of support treatment used in cases of side effects (probiotics, prokinetics, anti-acids), accounts for a further 5-25 € on average. In cases of therapeutic failure the patient will undergo a new medical examination (average cost: 36 €) and new surveys for *H. pylori* status by non-invasive (urea breath test: 70 €, stool antigen: 30-40 €) and invasive methods (esophagogastroduodenoscopy: 220 + 36 € of individual contribution; biopsy: 40 €; histological examination: 95 + 36 € of individual contribution)^[29].

In this scenario it is clear that inappropriate use of an anti *H. pylori* regimen incurs heavy costs not only in terms of the initial, ineffective treatment but also of the later additional visits and investigations.

FUTURE PERSPECTIVES

In future it will be more effective to test for clarithromycin resistance before starting conventional triple therapy. The limit of this approach is the high cost of molecular analysis, as well as the difficulty of performing phenotypic investigations, even for experts. Other issues hindering a wider spread of these assessments in clinical practice are the long time between testing and obtaining the results on which to plan therapy, and the need to perform an invasive examination (esophagogastro-duodenoscopy). In conclusion, it would be very useful to be able to test for clarithromycin resistance before starting conventional triple therapy. Hopefully, fast, effective non-invasive tests may soon be devised to determine this condition.

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