Usefulness of antimicrobial susceptibility in the eradication of *Helicobacter pylori*

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Abstract

The rate of eradication of *Helicobacter pylori* with standard triple therapy using omeprazole, amoxicillin and clarithromycin (OAC) is unacceptable in populations with high rates of clarithromycin resistance (15–20%). The aim of this study was to compare the efficacy of 10-day OAC therapy as the first-line treatment in patients diagnosed by culture with antimicrobial susceptibility or diagnosed by a ^{13}C-labelled urea breath test (UBT) without antimicrobial susceptibility in an area where the clarithromycin resistance rate was 15–20%. This was a retrospective cohort study of 266 patients, recruited consecutively throughout 2008. A total of 247 *H. pylori*-infected patients received antibiotic therapy (221 received the 10-day OAC therapy and 26 received other regimens) of which 134 patients were diagnosed by culture of gastric samples followed by antimicrobial susceptibility testing and 113 were diagnosed by UBT. In all patients, the eradication of *H. pylori* was checked by UBT. The cost of eradication by 10-day OAC treatment was assessed in each patient. The success rate of 10-day OAC therapy in patients diagnosed by culture and by UBT was 88% (103/117) and 49% (51/104), respectively ($p < 0.0005$). The treatment was also more cost-effective in the former of these two groups (€571 versus €666). To perform culture and antimicrobial susceptibility of the *H. pylori* isolates was a more successful and cost effective strategy than empirical 10-day OAC treatment in populations with high rates of resistance to clarithromycin.

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Introduction

The prevalence of *Helicobacter pylori* infection ranges from 30% to 50% in developed countries and from 70% to 80% in developing countries [1]. Some individuals in the infected populations will develop chronic atrophic gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and even gastric adenocarcinoma. The treatment of gastrointestinal diseases related to *H. pylori* infection is routinely required in clinical practice.

Standard triple therapy, a combination of a proton pump inhibitor with two antibiotics (clarithromycin + amoxicillin or metronidazole) for 7 days is only recommended for the treatment of *H. pylori* infections in geographical areas where the rates of resistance to clarithromycin are below 15–20% or where resistance to metronidazole is below 40% [2]. In the regions where the rate of resistance to clarithromycin is >20% alternative eradication regimens have been proposed, such as the sequential triple therapy, bismuth quadruple therapy, and concomitant quadruple therapy, based on omeprazole plus three of the following antibiotics: clarithromycin, amoxicillin, metronidazole, tetracycline or bismuth, or alternatively testing antimicrobial susceptibility of the *H. pylori* isolate before treatment [3].

The appropriate use of antibiotics requires the prescription of regimens that are sufficient to optimize the eradication of

H. pylori, maximizing their efficacy, while avoiding prolongation of treatment beyond the period necessary to eradicate the bacteria. For this reason, it is recommended that reference laboratories monitor the primary resistance, performing culture and antimicrobial susceptibility testing, to establish which therapies will achieve the higher eradication rates in each area. In our region, the Basque Country (Spain), resistance to clarithromycin was >15% in the last 5 years. The objective of the present study was to assess the usefulness of antimicrobial susceptibility in the eradication of *H. pylori*.

TABLE 1. Demographic and clinical characteristics of patients enrolled in the study

Variable	Diagnostic method		p
	Culture with antibiogram	UBT without antibiogram	
No. of patients	134	113	
Sex, male/female	71/63	50/63	0.2
Age (years) mean ± SD	48.3 ± 14.4	48.1 ± 14.2	0.93
Indication, *n* (%)			
Dyspepsia	72 (53.7)	58 (51.3)	0.8
Peptic ulcer disease	49 (36.5)	38 (33.6)	0.7
Gastric cancer in first-degree relatives	11 (8.2)	17 (15.1)	0.1
Gastric MALT lymphoma	2 (1.6)	–	0.5

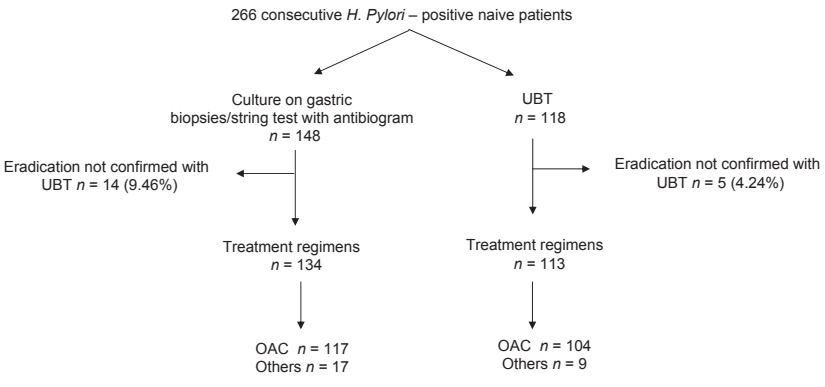
UBT, ^{13}C urea breath test; MALT, mucosa-associated lymphoid tissue.

Patients and Methods

This was a retrospective cohort study of 266 patients, recruited consecutively throughout 2008 when they attended the Donostia Hospital, in San Sebastian (Basque Country) or any of three specialist care centres under the management of the Donostia Hospital. All these patients were diagnosed with *H. pylori* infection through culture of gastric biopsy or string test followed by antimicrobial susceptibility testing (148 cases) or by ^{13}C-labelled urea breath test (UBT; 118 cases; Fig. 1). Patients had dyspeptic symptoms, peptic ulcer disease or first-degree family history of gastric cancer or gastric mucosa-associated lymphoid tissue lymphoma (Table 1). The exclusion criteria were being younger than 18 years old, having any severe concomitant disease, having previously undergone *H. pylori* eradication treatment or gastric surgery, or intolerance to any antimicrobial drugs.

Oral gastroscopies to obtain biopsies of the gastric body and antrum were performed depending on the digestive signs and symptoms following the recommendations of the specialist. The string test consisted of a capsule attached to a highly absorbent nylon string, which is swallowed to obtain gastric secretions using a procedure previously validated by our group [4,5]. Antimicrobial susceptibility against metronidazole, clarithromycin, levofloxacin, tetracycline and amoxicillin was measured using the E-test (bioMérieux SA, Marcy l'Etoile, France). Overall, during 2008, 437 *H. pylori* isolates were tested for antimicrobial susceptibility at the Microbiology Department of the Donostia Hospital (Table 2). For the UBT, a 100-mg UBTest (Otsuka Pharmaceutical Europe, Barcelona, Spain) was used. Breath samples were analysed using a mass spectrophotometer (NDIRS, Otsuka Pharmaceutical) and were considered positive when the value of ^{13}C (difference between baseline and post values) was over 2.5 delta units (>2.5%).

Patients diagnosed by culture (biopsy or string test) were treated with a regimen based on the antimicrobial susceptibility. In 117 patients infected with an isolate sensitive to clarithromycin, the therapy used was 10-day OAC (a twice daily combination of omeprazole, 20 mg; amoxicillin, 1 g and clarithromycin, 500 mg for 10 days). When resistance to clarithromycin was detected (17 cases), the classical quadruple treatment with bismuth (OBMT; omeprazole/bismuth/metronidazole/tetracycline) for 10–14 days and four different 10-day triple therapies were used, based on antimicrobial susceptibility. In these four triple regimens the clarithromycin



UBT: 13_C-urea breath test (100 mg UBT Otsuka), O, omeprazole; A, amoxicillin; C, clarithromycin

FIG. 1. Flow diagram of subject progress through the of study phases.

TABLE 2. Percentage of *Helicobacter pylori*-resistant isolates in Gipuzkoa (Basque country) 2006–10

Year	2006	2007	2008	2009	2010
No. of isolates	350	383	437	677	770
Metronidazole	32.0	38.8	37.2	37.2	41.4
Clarithromycin	15.7	17.8	21.1	17.6	18.1
Levofloxacin	11.7	19.3	15.1	16.1	16.6
Tetracycline	0	0	0	0.6	0.4
Amoxicillin	0	0	0	0	0

of the standard triple therapy was swapped for levofloxacin (500 mg), metronidazole (500 mg), rifabutin (150 mg) or doxycycline (100 mg), all given twice daily (OAL, OAM, OAR and OAD, respectively).

Patients diagnosed by UBT (113 cases) were treated empirically, with the first-line eradication triple therapy 10-day OAC (in 104 cases) but in nine cases other regimens: OAM, OAL, OML (twice daily omeprazole, 20 mg; metronidazole, 500 mg and levofloxacin, 500 mg for 10 days) or OMC (twice daily omeprazole, 20 mg and clarithromycin, 500 mg plus thrice daily metronidazole, 500 mg for 10 days) were used at the discretion of the gastroenterologist (Table 3).

To confirm the eradication of *H. pylori* the UBT was performed 6–8 weeks after the end of the treatment in all patients. The definitive results, as well as adherence to therapy and any adverse effects of the treatment, were assessed during patient follow-up visits.

Statistical analysis

To compare the differences between the two groups, the Fisher test was used for qualitative variables and the Student's *t*-test for quantitative variables. Differences were considered to be significant when the p values were below 0.05.

Cost analysis

To estimate the cost of the eradication of *H. pylori*, the direct costs of visits to the doctor, diagnostic methods and antimicrobial drugs were assessed. Costs of the first consultation (€110), follow-up visit (€50), gastroscopy with biopsy (€202.20), antimicrobial susceptibility test (€40), string test (€22), the breath test (€46.50) and the antimicrobial drugs (€25) were taken from the 2008 price list of the institution (Donostia Hospital).

Results

Of the 266 patients initially included, 19 were excluded because confirmation of eradication was not performed (14 in the group of patients with an antibiogram and five in the UBT group), and 247 (121 men and 126 women) were finally included (Fig. 1). These exclusions do not interfere with the results. The rate of success of the 10-day OAC therapy in patients diagnosed by culture with clarithromycin susceptibility was 88% (103/117) while that in those treated with 10-day OAC and diagnosed by UBT was 49% (51/104) ($p < 0.0005$). When all initial treatment regimens were considered, the rates of eradication were 84.3% (113/134) and 50.4% (57/113) for patients diagnosed by culture with antimicrobial susceptibility or by UBT, respectively ($p < 0.0005$; Table 3).

Among the 14 patients in the group diagnosed by culture with antimicrobial susceptibility, in whom eradication was not achieved by OAC, four cases of resistance to metronidazole and two to levofloxacin were detected. Among the 17 patients with resistance to clarithromycin, who received other types of eradication regimens according to the antimicrobial susceptibility, isolates obtained in 11 patients also showed resistance to metronidazole (seven patients), levofloxacin (two patients) or both (two patients).

In 30 of the 53 patients diagnosed by UBT in whom eradication was not achieved with 10-day OAC, a second treatment was given based on various different regimens (19 with

TABLE 3. Efficacy of *Helicobacter pylori* eradication with different treatment therapies

Diagnostic method					
Culture with antibiogram (*n* = 134 patients)			UBT without antibiogram (*n* = 113)		
Treatment therapies	No. patients	No. eradication	Treatment therapies	No. patients	No. eradication
OAC	117	103[a]	OAC	104	51[a]
OAL	6	3	OAM	5	5
OAM	4	3	OAL	1	0
OBTM	3	3	OML	1	0
OAD	3	1	OMC	2	1
OAR	1	0			
	134	113[b]		113	57[b]

[a]p (103 vs. 51) <0.0005.
[b]p (113 vs. 57) <0.0005.
O, omeprazole; A, amoxicillin; C, clarithromycin; L, levofloxacin; M, metronidazole; B, tripotassium dicitrato bismuthate; T, tetracycline; D, doxycycline; R, rifampicin.

OAL, five with OAM, five with OBMT and one re-treated with OAC). With these second treatments, eradication was achieved in 24 of the 30 (80%) cases; with treatment success in 14 of the 19 (73.6%) patients treated with OAL.

Some adverse effects were observed in 31 (14%) of the 221 total patients treated with 10-day OAC (11 cases of altered taste, eight of diarrhoea, six of stomach ache, four of headache and two of oral candidiasis), nonetheless all patients completed the full treatment.

The average cost of the eradication with 10-day OAC was €571.20 per patient when diagnosis was made by culture with antimicrobial susceptibility, and €666.80 in the UBT group, where patients were empirically treated.

Overall, in 2008, *H. pylori* resistance rates were 37.2% for metronidazole (MIC >4 mg/L), 21.1% for clarithromycin (MIC >1 mg/L according to the recommendation of the CLSI [6]) and 15.1% for levofloxacin (MIC >2 mg/L). No resistance was detected to tetracycline (MIC >4 mg/L) or amoxicillin (MIC <1 mg/L). A total of 51.2% of all isolates were resistant to at least one type of antibiotic, and 14.9% of the isolates were multi-resistant. Notably, the rate of the double resistance to clarithromycin and metronidazole was 8.9%.

Discussion

The commonest cause of failure to eradicate *H. pylori* is antibiotic resistance and this has increased, particularly resistance to clarithromycin. In the Basque Country (Spain), from 1994 to 1998, considering 1419 *H. pylori* isolates, the primary resistance to clarithromycin was 10.7% (rising year on year: 5.6% in 1994, 7% in 1995, 8.5% in 1996, 12.9% in 1997 and 19.5% in 1998) [7]. In the 5-year period 2006–10, in a total of 2617 *H. pylori* isolates the primary resistance to clarithromycin reached 18% (15.7% in 2006, 17.8% in 2007, 21.1% in 2008, 17.6% in 2009 and 18.1% in 2010; Table 2). In Europe, primary resistance to this antibiotic in adults has a north/south gradient, with prevalence being lower in northern European countries (2–11%) and higher in the south (12–22%) [8,9].

Across Europe [9], around 10% of the *H. pylori* isolates are resistant to more than one family of antibiotics. In our region, multi-resistance was detected in 10.5% of the *H. pylori* isolates in the period 1994–98 and in 14.9% in 2008. Double resistance to clarithromycin and metronidazole was 8.9% in the present study, higher than the figure cited in a recent literature review [9], where the highest rate was that for Japan (6.6%). This situation makes it even more difficult for *H. pylori* eradication treatments to be successful and increases the need to search for other therapeutic strategies.

In two European multicentre trials [10,11] (MACH1 and MACH2), reported in 1996 and 1999, the efficacy of the 7-day OAC and OMC triple therapies for the eradication of *H. pylori* was confirmed. Notably, in the MACH2 study the rate of resistance to clarithromycin was just 3%. At the European Helicobacter Study Group (EHSG) meeting [12], held in Maastricht (1997), the standard triple therapy (7–10 days of OAC) was recommended as the first-line treatment for *H. pylori* infection (first consensus conference). Since then, the eradication success according to intention-to-treat (ITT) analysis has fallen from 90% to 75–80% in regions with high rates of resistance to clarithromycin [8,13]. In 2010, Graham and Fischbach [14], reviewed 121 studies on the efficacy of the first-line of treatment in adults carried out between 1997 and 2009, and demonstrated that only 18% of adults have more than an 85% chance of the infection being successfully eradicated (on the basis of ITT analysis). Moreover, in 60% of these studies the success of eradication of *H. pylori* did not reach 80% (again, with ITT analysis). Some of these studies were carried out in populations of Central and Southern Europe with resistance rates to clarithromycin >18%.

To increase the eradication rates with standard triple therapy (OAC) it has been suggested that the dose of proton pump inhibitors or the duration of treatment be increased. Villoria *et al.* [15]. reported that the rate of eradication with OAC treatments increases by 8% if the dose of omeprazole is doubled with respect to that normally used and Fuccio *et al.* [16] reported an increase in the eradication of 4–5% when the duration of the treatment is 10–14 days, instead of 7 days. The recommended duration of the triple OAC is 10–14 days in the USA [17] whereas it is 7 days in Europe [2]. Nonetheless, it is accepted that with a prevalence of resistance to clarithromycin higher than 15–20%, a standard triple therapy, including macrolides, should not be used on an empirical basis.

The inappropriate use of antibiotics in the community, together with population migration, has changed the levels of resistance within countries, and also within small geographical areas. This makes it necessary to search for treatment regimens that achieve 90% eradication, according to ITT analysis, in specific local communities knowing the local levels of antibiotic resistances, rather than following recommendations in general guidelines and consensus. To date, the effectiveness of various empirical eradication therapies has been confirmed, including the sequential triple therapy (omeprazole 20 mg + amoxicillin 1 g twice daily given for the first 5 days followed by a triple therapy including omeprazole 20 mg, clarithroycin 500 mg and metronidazole 500 mg, all twice daily, for the remaining 5 days), the classical bismuth-containing quadruple therapy (omeprazole 20 mg twice daily + bismuth salt 120 mg, tetracycline 500 mg and metro-

nidazole 250 mg, four times a day, for 10–14 days) and concomitant quadruple therapy (triple therapy OAC + metronidazole 500 mg, all twice daily for 10 days) as Graham and Fischbach have stated [14].

In 2004, Mégraud [18] reviewed several clinical trials published between 1999 and 2003 to assess the efficacy of the standard triple therapy (OAC) based on the *H. pylori* antibiotic sensitivity. The study that included 1975 isolates showed that the rate of eradication using the OAC triple treatment decreased from 87.8% to 18.3%, when *H. pylori* were susceptible or resistant to clarithromycin. In the same study, with 656 isolates analysed, the eradication efficiency of the OAM triple regimen decreased by 25% (from 89.4% to 64.4%) when *H. pylori* isolates were susceptible or resistant to metronidazole.

The poor efficacy of the empirical OAC triple therapy in the eradication of *H. pylori* when the prevalence of clarithromycin resistance is higher than 20% represents a challenge for clinicians and it was the reason for the present study. Our findings indicate that this problem, compounded by the existence of multi-resistance to various families of antibiotics and the increase in resistance to other antimicrobial drugs [9,19], may be addressed by the appropriate use of antibiotics, in a less costly way and with courses kept as short as possible. Furthermore, high rates of clarithromycin resistance (around 50%) are always present in cases when clarithromycin-based first-line treatment has failed. This means that in such a setting susceptibility testing would always be mandatory.

To perform antimicrobial susceptibility before deciding on a definitive treatment is essential in populations with high rates of resistance to clarithromycin such as ours, because the eradication rates are improved and treatments are more cost-effective.

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Transparency Declaration

The authors declare no conflicts of interest.

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