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Short Communication

# Clarithromycin resistance in *Helicobacter pylori* and its molecular determinants in Northern Spain, 2013–2015

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ABSTRACT

*Objectives:* Clarithromycin resistance (CLR-R) is the main reason for failure of *Helicobacter pylori* infection treatment, which is frequently empirically prescribed due to the erroneous belief that culture for susceptibility testing is difficult. The aim of this study was to determine CLR-R in a region of southern Europe and to evaluate the utility of a PCR sequencing assay applied on gastroduodenal biopsies in detecting *H. pylori* and clarithromycin (CLR) susceptibility.
*Methods:* The susceptibility of all *H. pylori* isolates obtained by culture during 2013–2015 was determined by Etest. During 2014–2015, *H. pylori* detection and CLR susceptibility were also studied by PCR followed by sequencing performed on gastroduodenal biopsies. Point mutations in the 23S rRNA gene were studied in all CLR-resistant isolates in 2014.
*Results:* Of 1986 *H. pylori* isolates obtained by culture (63 from children and 1923 from adults), 349 (17.6%) were CLR-resistant [21/63 (33.3%) in children and 328/1923 (17.1%) in adults; $P < 0.001$], of which 31.5% were also resistant to levofloxacin. The main mutations detected were A2147G (79.8%), A2146G (17.2%) and A2146C (2%). Concordance between the PCR sequencing assay on biopsies and CLR susceptibility by Etest after culture was 89.8%.
*Conclusions:* CLR-R was high in Gipuzkoa, northern Spain. The molecular PCR method performed directly on biopsies was a good alternative to the traditional Etest susceptibility method and was an aid when culture was non-viable.


## 1. Introduction

Clarithromycin resistance (CLR-R) is the main factor in failure of *Helicobacter pylori* eradication treatment. Empirical standard triple therapy, based on a proton pump inhibitor and clarithromycin (CLR) with amoxicillin or metronidazole (MTZ), is recommended in most guidelines in populations with a CLR-R rate below 15–20% or a MTZ resistance rate below 40% [1]. In the last decade, CLR-R has increased in many countries [2] and suboptimal eradication rates have been repeatedly reported following standard triple therapy [3].

CLR-R in *H. pylori* is mainly associated with mutations within the peptidyl transferase loop region in domain V of 23S rRNA [4,5]. Three major point mutations have been described in the 23S rRNA gene: A2146C, A2146G and A2147G (the numeration is from genome sequencing of NC000921 and NC000915, positions 2146 and 2147, formerly described as 2142 and 2143) [6].

The aims of this study were: (i) to determine *H. pylori* CLR-R rates among children (age <15 years) and adults; (ii) to

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**Table 1**
Clarithromycin (CLR) resistance and associated resistance to levofloxacin (LVX) and metronidazole (MTZ) in *Helicobacter pylori* isolates obtained by culture during 2013–2015, Gipuzkoa, Northern Spain.

| Year | No. of isolates studied | | | CLR-resistant isolates [*n* (%)] | | | Associated resistance to LVX | | | Associated resistance to MTZ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Children | Adults | Total | Children | Adults | *n* | % of total | % of CLR-resistant | *n* | % of total | % of CLR-resistant |
| 2013 | 398 | 18 | 380 | 75 (18.8) | 7 (38.9) | 68 (17.9) | 23 | 5.8 | 30.7 | 35 | 8.8 | 46.7 |
| 2014 | 654 | 14 | 640 | 99 (15.1) | 5 (35.7) | 94 (14.7) | 36 | 5.5 | 36.4 | 45 | 6.9 | 45.5 |
| 2015 | 934 | 31 | 903 | 175 (18.7) | 9 (29.0) | 166 (18.4) | 51 | 5.5 | 29.1 | 64 | 6.9 | 36.6 |
| Total | 1986 | 63 | 1923 | 349 (17.6) | 21 (33.3) | 328 (17.1) | 110 | 5.5 | 31.5 | 144 | 7.3 | 41.3 |

characterise the molecular determinants in CLR-resistant isolates; and (iii) to evaluate a PCR sequencing assay to detect *H. pylori* and its susceptibility to CLR on gastroduodenal biopsies in order to favour a tailored treatment without culture.

## 2. Materials and methods

This study was conducted at Hospital Universitario Donostia (San Sebastián, Gipuzkoa, Spain) and was approved by its Ethic Committee. All gastroduodenal biopsies received by the microbiology department of Hospital Universitario Donostia for *H. pylori* culture during 2013–2015 were included in the prevalence study of resistance. The primary resistance rate was calculated in a sample of 91 *H. pylori* isolates obtained during 2014–2015from adults who had never previously been treated for this infection.

### 2.1. Susceptibility testing by Etest

*Helicobacter pylori* isolates were obtained from gastroduodenal biopsies cultured on selective plates (Pylori Agar; bioMérieux SA, Marcy-l'Étoile, France). Plates were incubated under microaerophilic conditions at 37 °C with 80% humidity for 7 days before discarding them as negative. Antimicrobial susceptibility testing was performed using Etest strips (bioMérieux SA) on *Brucella* agar plates supplemented with 5% haemolysed horse blood and 1% Vitox (Oxoid Ltd., Basingstoke, UK). Plates were incubated for 72 h under the same conditions described above.

Isolates with minimum inhibitory concentrations (MICs) for CLR, levofloxacin (LVX) and MTZ of ≥1, ≥2 and >8 mg/L, respectively, were considered CLR-, LVX- and MTZ-resistant.

### 2.2. 23S rRNA mutations of clarithromycin-resistant Helicobacter pylori isolates

Mutations in the 23S rRNA gene were studied in all CLR-resistant *H. pylori* isolates obtained by culture in 2014 ($n = 99$). Isolates were digested with proteinase K for ≥1 h before DNA extraction using NucliSENS® easyMag® (bioMérieux, SA). A 267-bp fragment of the 23S rRNA gene was amplified and sequenced using primers (HPY-S and HPY-A) described by Ménard et al. [7] with the following conditions: 40 amplification cycles consisting of a denaturation step at 94 °C for 1 min, an annealing step at 57 °C for 30 s and an extension step at 72 °C for 1 min. In CLR-resistant strains without mutations at positions 2146 and 2147, a longer fragment of 884 bp (nt 1934-2817) of the 23S rRNA gene was amplified using the primers HPY-S and HP2R (5′-TGTGTGCTACCCAGCGATGCTC-3′; this study) with the following conditions: 35 amplification cycles consisting of a denaturation step at 94 °C, an annealing step at 64 °C and extension step at 72 °C, each for 1 min.

To search for point mutations, sequences were compared with the 23S rRNA gene of the CLR-susceptible *H. pylori* strain NGY186-S (GenBank accession no. AB162858.1).

### 2.3. Detection of H. pylori DNA and molecular clarithromycin susceptibility in gastroduodenal biopsies

The same biopsy after being cultured on selective plates was used to perform the direct detection of *H. pylori* and CLR susceptibility using the PCR sequencing assay described above. Biopsies were digested with proteinase K for ≥4 h before DNA extraction using NucliSENS® easyMag®.

Concordance of CLR susceptibility between Etest (in the culture isolate) and the direct PCR sequencing assay (in biopsy) was assessed by testing 1358 biopsies positive for *H. pylori* obtained during 2014–2015 that simultaneously underwent the two tests (228 CLR-resistant and 1130 CLR-susceptible).

## 3. Results

During the study period, 6228 gastroduodenal biopsies were tested, yielding 2116 (34.0%) positive cultures for *H. pylori*. Antimicrobial susceptibility was available in 1986 (93.9%) of the 2116 isolates. The overall CLR-R rate of the 1986 *H. pylori* isolates (1923 from adults and 63 from children) obtained during 2013–2015 was 17.6% (349/1986), without no significant difference between years. CLR-R was significantly higher in children compared with adults [33.3% (21/63) vs. 17.1% (328/1923); $P < 0.001$]. The primary CLR-R rate in adults was 7.7% (7/91).

Among the 349 CLR-resistant isolates, 110 (31.5%) showed associated resistance to LVX and 144 (41.3%) to MTZ (Table 1). A total of 47 CLR-resistant isolates (13.5%) showed multiresistance, including resistance to both LVX and MTZ.

All CLR-resistant isolates detected in this region during 2014 harboured a mutation in the 23S rRNA: 99.0% (98/99) harboured one of the three main mutations, with the most common being A2147G (79.8%), followed by A2146G (17.2%) and A2146C (2%), all of them with a CLR MIC of >250 mg/L. The only other mutation found was C2699A, which was detected in one isolate with a CLR MIC of 1 mg/L.

The correlation between culture and PCR for *H. pylori* detection was assessed in 4256 gastric biopsies, showing a concordance rate of 95.7% (Table 2).

Concordance in CLR susceptibility between the PCR sequencing method and Etest was 89.8%, specifically 71.5% (163/228) in biopsies with CLR-resistant isolates and 93.5% (1056/1130) in biopsies with CLR-susceptible isolates (Table 3). During

**Table 2**
Correlation between culture and PCR for *Helicobacter pylori* detection among 4256 gastric biopsies in Gipuzkoa, Northern Spain, 2014–2015.

| Culture | Biopsy PCR | | Concordance |
|---|---|---|---|
| | Positive | Negative | |
| Positive ($n = 1572$) | 1487 | 85 | 94.6% (1487/1572) |
| Negative ($n = 2684$) | 97 | 2587 | 96.4% (2587/2684) |