Characterization of clarithromycin resistance in isolates of *Helicobacter pylori* from the UAE

Mubarak S. Alfaresi · Abida A. Elkoush

Indian J Gastroenterol (2010) 29:116–120
DOI 10.1007/s12664-010-0034-z

**Abstract**

*Background* Clarithromycin therapy is effective in eradicating *Helicobacter pylori*. However, the resistance of *H. pylori* to clarithromycin is increasingly reported. The present study aimed to characterize the types of mutations present in the 23S rRNA genes of isolates of clarithromycin-resistant *H. pylori* from the UAE.

*Methods* Clarithromycin susceptibility of *H. pylori* isolates (*n* = 26) was determined by E tests. Analyses for point mutations in domain V of the 23S rRNA genes in clarithromycin-resistant and-sensitive strains were performed by sequence analysis of amplified PCR products.

*Results* Out of 100 gastric antral biopsy samples, 26 were positive for *H. pylori* by culture, and 29 were positive by PCR. Of the 26 culture isolates, five (19.2%) were resistant to clarithromycin and 24 were sensitive. The MIC of the resistant strains ranged from 3 to 24 μg/mL (median 24). All of the clarithromycin-resistant isolates had point mutations in the 23S rRNA gene. Two isolates had an A2142G 23S rRNA mutation, and three had A2143G mutations.

*Conclusion* Clarithromycin resistance was common in this small collection of *H. pylori* isolates from the UAE. The A2142G and A2143G mutations were associated with clarithromycin resistance.

**Keywords** Drug resistance · Rapid urease test

**Introduction**

*Helicobacter pylori* (*H. pylori*) is a microaerophilic, Gram-negative bacterium and is implicated as a causative agent of chronic gastritis, duodenal and gastric ulcers, and gastric carcinoma [1, 2]. Infection with *H. pylori* can be effectively treated by a combination of a proton pump inhibitor with multiple antibiotics. The first-line regimen consists of triple therapy, with clarithromycin as one of the most widely used components. Although the bacteria can be eradicated in up to 90% of patients, side effects, poor compliance, and resistance to the antibiotics used are common causes of treatment failure [3, 4]. Unfortunately, the increasing use of clarithromycin has resulted in the development of resistance. The prevalence of resistant strains varies among countries and ranges from 1% in Norway [5] to 29% in Japan [6]. The mechanism of resistance to clarithromycin in *H. pylori* seems to be a decrease in binding of macrolides to the ribosome, associated with point mutations within the peptidyltransferase-encoding region of the 23S rRNA gene [7, 8]. Three major point mutations, in two positions, have been described in which an adenine residue is replaced by a guanine or a cytosine: A2142C, A2142G, and A2143G [7–10].

In the present study, we determined the prevalence of clarithromycin resistance in our local *H. pylori* strains and characterized the types of mutations that occurred in the resistant isolates.
Methods

Sample collection

From July 2008 to January 2009, a total of 100 gastric biopsy samples were obtained from dyspeptic patients referred to the endoscopy departments in Zayed Military Hospital (ZMH) in Abu Dhabi, UAE (mean age 40 years [range 19–80]; 70 male). For each patient, two endoscopic biopsy specimens were taken from the antrum of the stomach. One sample was initially tested for *H. pylori* infection with a CLO test at the endoscopy unit in ZMH. The other sample was divided into two; the first part was used for culture and the second part was directly frozen at −20°C for molecular analysis. The research was conducted according to appropriate ethical guidelines and approved by the ethical committee at ZMH.

*H. pylori* culture and antimicrobial susceptibility determination

Biopsy samples were cultured on Columbia agar supplemented with 10% ox blood. Plates were incubated at 37°C under microaerophilic condition for 5–7 d. Bacterial isolates were identified according to colony morphology, Gram-staining, urease, catalase and oxidase activities.

The MIC of clarithromycin was determined by the E-test method. E tests (AB Biodisk, Sweden) were performed on Columbia agar supplemented with 10% ox blood. The plates were incubated under microaerophilic condition for 3–5 d. Isolates were classified as clarithromycin-resistant if the MIC was >1 µg/mL. *H. pylori* strain ATCC43504 was included as a control clarithromycin-sensitive strain.

PCR identification of *H. pylori* in biopsy samples

Identification of the organism in biopsy samples was also carried out using PCR with primers (5′ to 3′) (Table 1) specific for *H. pylori* [11] and visualization of a 298-bp fragment on agarose gel stained with ethidium bromide. The primer pair was based on the DNA sequence of a species-specific antigen reportedly present in all strains of *H. pylori* tested [11]. DNA was isolated from 25 to 50 mg thawed biopsy tissue using a QIAamp DNA Mini kit (QIAGENE, Cat No: 51306) according to the manufacturer’s direction. The isolated DNA was eluted in 60 µL elution buffer and stored at −20°C until further analysis. The amplification of DNA was done in 0.2 ml reaction tubes by PCR using a thermal cycler (Perkin Elmer 2400). Reaction mixtures (50 µl) consisted of 1 × PCR master mix (Qiagene, Cat No: 201445), forward and reverse primers each at a concentration of 0.1 µM, and 2 µl of the extracted DNA as a template, where the best band resolution was in the concentration range from 100 to 200 ng/µl. Each reaction mixture was amplified as follows (Table 2): denaturation at 94°C for 5 min, and then the sample was allowed to undergo 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 68°C, and extension for 1 min at 72°C. A final extension was carried out for 10 min at 72°C to ensure complete amplification. Sequenced samples from positive amplified PCR products served as positive controls, whereas distilled water served as a negative control during the amplification. The amplified DNA product was analyzed by electrophoresis using agarose (Invitrogen, Cat No: 10975-035) prepared in TBE (Tris Borate-EDTA) buffer (1.5 g/100 ml). The size of the gel was 10×7 cm with a thickness of 0.3 cm. Samples were loaded on the gel, and a current of 40 mA was applied. A 100 bp DNA ladder (Invitrogen, Cat No: 10488-058) was used as a molecular weight marker. The sample was allowed to run for 2 h, and the gel was stained with 0.5 mg/ml ethidium bromide for 30 min. The band was seen by illumination with UV light in a gel documentation system.

Detection of point mutations in the 23S rRNA gene of *H. pylori* by sequencing

23S RNA was directly amplified from 26 biopsies from which the organism had been cultured and MIC obtained. PCR-positive samples that failed to be cultured were excluded from this study because MIC could not be determined in the absence of culture. Point mutations at

<table>
<thead>
<tr>
<th>Table 1 Primer sequences used in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Species-specific antigen gene</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>CLA18:</td>
</tr>
<tr>
<td>5′-AGTCGGGACCTA</td>
</tr>
<tr>
<td>AGGCGAG-3′</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 PCR programs to amplify different genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Species-specific antigen gene</td>
</tr>
<tr>
<td>23s rRNA gene</td>
</tr>
</tbody>
</table>
two positions in the 23s rRNA gene (2142 and 2143) were identified by amplifying 1,400 bp of the target gene encoding 23S rRNA and then sequencing it [12]. PCR amplifications were done using oligonucleotide primers CLA18 and CLA21 (Table 1), which are complementary to conserved regions of the 23S rRNA gene. Each PCR was performed in a volume of 50 µl containing 1 × PCR master mix, forward and reverse primers each at 1 µM, and 2 µl of the extracted DNA. Each reaction mixture was amplified as follows (Table 2): denaturation at 94°C for 6 min and then 40 cycles of 94°C for 30 s, 50°C for 1 min, and 72°C for 3 min. A final extension was carried out for 10 min at 72°C to ensure complete amplification. PCR products were purified with a QIAquik purification kit (Qiagen), and a cycle sequencing reaction was performed with the same primers.

Only PCR-positive samples for the 23S rRNA gene of *H. pylori* were cultured and sequenced. Sequencing was performed using a BigDye Terminator v3.1 sequencing kit and analyzed on an ABI PRISM 377 Genetic Analyzer. The sequences were analyzed and aligned using Geneiuos software.

**Statistical analysis**

SPSS software (Version 18, SPSS Inc., Chicago, Illinois, USA) was used to calculate chi-squared to test for differences in proportions between the two groups. P values less than 0.05 were reported as statistically significant.

### Results

A total of 100 gastric biopsy samples were subjected to CLO test, bacterial culture, DNA extraction and PCR amplification of the 23S rRNA gene. Twenty-two (22%) samples were positive for CLO test. Twenty-six (26%) samples were positive for *H. pylori* by bacterial culture. Twenty-nine (29%) samples were positive for *H. pylori* by PCR. All CLO positive samples were also positive by culture and PCR methods. Five (19.2%) of these positive culture isolates were resistant to clarithromycin by antimicrobial susceptibility E-tests, while 21 isolates were sensitive. The MIC values of the clarithromycin-resistant isolates ranged from 3 to 24 µg/mL (Table 3). The mutations in domain V of the 23S rRNA were determined by comparing the sequences of clarithromycin-resistant and -sensitive isolates with the published *H. pylori* 23S rRNA gene reference sequence (Gene Bank accession number U27270) (Fig. 1). All of the clarithromycin-resistant isolates were shown to have point mutations of either A2142G or A2143G, and none of the 21 clarithromycin-sensitive isolates had these mutations. Three isolates (60%) contained the A2143G mutation, and the A2142G was present in the other two (40%) (Table 3). All clarithromycin-resistant strain sequences were deposited in the Gene Bank (GU120184-GU120188).

### Discussion

The prevalence of clarithromycin resistance was found to be high among the *H. pylori* isolates in the present study. The prevalence of *H. pylori* resistance to clarithromycin varies among different countries, being 12% in Japan, 1.7–23.4% in Europe and 10.6–25% in North America [13, 14]. To the best of our knowledge, this was the second study that reported and characterized clarithromycin-resistant strains in the UAE and the first study using the 23S rRNA gene sequencing method.

Clarithromycin is used worldwide for *H. pylori* eradication therapy. *H. pylori* strains that are resistant to clarithromycin have been increasingly reported in several

---

**Table 3** MIC value of clarithromycin and mutations in the 23S rRNA domain V of resistant *H. pylori* isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Clarithromycin MIC (µg/mL) and phenotype</th>
<th>Mutation site</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP01</td>
<td>24 Resistant A2143G</td>
<td>GU120184</td>
<td></td>
</tr>
<tr>
<td>HP02</td>
<td>3 Resistant A2143G</td>
<td>GU120185</td>
<td></td>
</tr>
<tr>
<td>HP03</td>
<td>24 Resistant A2142G</td>
<td>GU120186</td>
<td></td>
</tr>
<tr>
<td>HP04</td>
<td>24 Resistant A2142G</td>
<td>GU120187</td>
<td></td>
</tr>
<tr>
<td>HP05</td>
<td>24 Resistant A2143G</td>
<td>GU120188</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1 Nucleotide sequence alignment of the 23S rRNA domain V in A2142G and A2143G mutant strains found in this study. The HPU27270 is the published sequence of the 23S rRNA gene of *H. pylori* (GeneBank accession number U27270)
studies [14, 15]. As resistance will often lead to failure of eradication therapy, knowledge of the current susceptibility patterns of local \textit{H. pylori} isolates could help in determining the choice of appropriate treatment for the patient.

The prevalence of mutant strains among the clarithromycin-resistant \textit{H. pylori} isolates varies in different parts of the world. Studies from the U.S.A. revealed 48 to 53\% with the A2142G mutation, 39 to 45\% with the A2143G mutation, and 0 to 7\% with the A2142C mutation [16, 17]. The prevalence of the A2142G mutation in Europe was reported as 23 to 33\%, the A2143G mutation as 44 to 67\%, and the A2142C mutation as 2 to 10\% [18, 19]. However, studies from Japan [20, 21] showed that more than 90\% of the mutant strains had the A2143G mutation, but the A2142C mutation was not detected. Although the number of the strains was small, a study from China also showed that 100\% of the clarithromycin-resistant \textit{H. pylori} isolates had the A2143G mutation [22].

Versalovic et al [23] have shown that the 23S rRNA gene A2142G and 2143G mutations in \textit{H. pylori} are associated with resistance to clarithromycin. A mutagenesis study performed by Taylor et al [24] has confirmed that the A2142G and A2143G mutations are associated with clarithromycin resistance. A review by Mégraud [13] of several studies worldwide demonstrated that 81.5\% of the mutations in clarithromycin-resistant isolates were the A2142G or A2143G mutations. In our study, either the A2142G or A2143G mutation was found in the clarithromycin-resistant isolates, and they were absent in the clarithromycin-sensitive isolates. The mutations found in our local strains were in accordance with the findings in other countries, and the mutations were also associated with low-level resistance. Mutations other than A2142G and A2143G have been reported in other studies [25, 26], but among our strains, sequence analysis showed that the mutations were limited to these types only. Although the low number of resistant isolates in our study limits the scope of detection, the results suggest that \textit{H. pylori} clarithromycin resistance in the UAE can be predicted by detection of mutations at positions 2142 and 2143 of the 23S rRNA gene.

In conclusion, we showed that there was high prevalence of clarithromycin resistance in our local \textit{H. pylori} strains. The A2142G or A2143G mutations detected were in accordance with the findings from other countries. These mutations were not found among the analyzed clarithromycin-sensitive strains. Other types of mutations associated with clarithromycin resistance were not observed in our resistant strains. These findings may have a significant impact on patient management, providing rapid information for the clinician, allowing, for example, appropriate antibiotic prescription and prediction of treatment outcome.

References