Molecular Approaches to Identify *Helicobacter pylori* Antimicrobial Resistance

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KEYWORDS

- Macrolides • Fluoroquinolones • Tetracyclines • Rifampins
- Polymerase chain reaction • Fluorescent in situ hybridization
- Real-time polymerase chain reaction • Dual priming oligonucleotide

KEY POINTS

- Detection of antimicrobial resistance of *Helicobacter pylori* is important to tailor the treatment and obtain the best outcome of eradication.
- Molecular methods that detect mutations in genes relevant to antimicrobial resistance can be applied, especially for the most important antibiotic (ie, clarithromycin).
- Numerous molecular methods have been proposed to detect the main 3 mutations associated with clarithromycin resistance of *H pylori*, the most commonly used being real-time polymerase chain reaction protocols.
- The correlation between molecular detection of resistance via mutations and antimicrobial susceptibility testing by Etest is not perfect, because the former is better for detecting heteroresistance, but which method correlates the best with eradication is not known.
- Molecular methods can also be applied to detect *H pylori* resistance to fluoroquinolones, tetracycline, and rifampin, although they are not so commonly used.
- The advantage of molecular methods is their rapidity, lack of stringent transport conditions, and standardization.
- Their limit is that they cannot be used for all antibiotics and they do not detect resistance caused by mutations other than those already known or other resistance mechanisms.

There are several reasons for failure of the treatments aiming to eradicate *Helicobacter pylori*. They include a poor compliance to the regimen and a high gastric acidity, which is not overcome by the recommended dose of proton pump inhibitor (PPI) that increases the minimal inhibitory concentration (MIC) of the antibiotics used. In the

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past, different conditions, such as an important bacterial load, infection by CagA (cytotoxin-associated gene A)-positive versus CagA-negative *H pylori* strains, and the presence of intracellular bacteria and some immunologic deficiencies have been suggested to influence eradication[1] but seem less important when susceptibility and compliance are taken into consideration.

H pylori may become resistant to all the antibiotics used for eradication in the various regimens proposed, essentially according to the same mechanism (ie, acquisition of point mutations).[2] Point mutations occur by chance, and increase the MIC of the bacteria. Those organisms with point mutations are then selected by the corresponding antibiotics when prescribed. Another mechanism that sometimes occurs is an efflux mechanism of resistance (ie, efflux pumps, which tend to eliminate the antibiotic having penetrated into the bacterial cell).

Acquisition of resistance in *H pylori* is important essentially for macrolides (clarithromycin) and fluoroquinolones (levofloxacin). It rarely occurs for β-lactams (amoxicillin), tetracyclines, and for rifampin (rifabutin). To the contrary, although they seem to be frequent for 5-nitroimidazoles (metronidazole), they can be overcome in vivo.[3]

As for any infection, it seems crucial to detect *H pylori* resistance before prescribing a treatment, the efficacy of which would be jeopardized by the presence of resistant organisms.

The standard detection method consists of performing an antibiogram, usually or MIC determination, using Etest. Although this procedure has the advantage of offering testing of all of the antibiotics of interest, it also has some drawbacks. It requires living organisms, and culturing *H pylori* is sometimes challenging because of the special transport conditions necessary for gastric biopsies, as well as special care in the laboratory; several days are necessary for primary culture and then performing the antibiogram. For these reasons, alternative methods to this phenotypic approach have been proposed, including various molecular approaches.

The aim of this article is to review these methods, focusing on the determination of *H pylori* resistance to macrolides and fluoroquinolones, which are the most important, and mentioning also the methods used for tetracycline and rifampin.

MOLECULAR DETERMINATION OF *HELICOBACTER PYLORI* RESISTANCE TO MACROLIDES

Mechanisms

Macrolides target the 23S ribosomal RNA (rRNA). There are in particular 2 nucleotide positions at the domain V level of the peptidyl transferase loop, which can lead to resistant organisms, because they induce a change in the ribosome conformation and decrease macrolide binding. These positions are 2142 and 2143. A transition can be found at both positions, whereas a transversion is found only at the former (**Fig. 1**).[4,5] Other mutations that could theoretically occur are not found in nature, possibly because they lead to nonviable organisms. Some reports of other mutations associated with clarithromycin resistance have been made but could not be confirmed.[6]

However, a recent study questions this dogma. Comparing phenotypic and genotypic resistance to clarithromycin, De Francesco and colleagues[7] found a high rate of discrepancy. Of 42 clarithromycin-resistant strains, only 23 harbored the 3 known mutations, whereas 19 did not. These investigators identified the following mutations in 14 of 19 cases: A2115G, G2141A, and A2144T.