**Helicobacter pylori** resistance to antibiotics: Prevalence, mechanism, detection. What’s new?

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**HELICOBACTER PYLORI RESISTANCE TO ANTIBIOTICS**

**Prevalence, mechanism, detection. What’s new?**


*Helicobacter pylori* resistance to antibiotics is increasingly reported and may limit the efficacy of current treatment regimens. Their resistance mechanism has been found to be point mutations for all antibiotics. Macrolide resistance is the most clinically important, but can be detected efficiently by molecular methods. Metronidazole resistance has limited clinical impact but testing methods are not reliable. Seldomly found cases of resistance, such as to amoxicillin and tetracycline, have had their mechanism recently elucidated. The existence of rapid and practical methods for the detection of macrolide resistance (eg, Fluorescence Resonance Energy Transfer assay) should improve management of *H pylori*-positive patients in the future, by allowing an adapted first-line therapy.

**Key Words:** Antibiotic resistance; Detection; Point mutations; Prevalence

As with any infectious disease, *Helicobacter pylori* infection must be treated with antibiotics. The experience gained during the last decade has shown that a combination of a proton pump inhibitor and clarithromycin-based therapies give the best cure rates, and these are now recommended in guidelines for the treatment of this infection (1-3). The most recent guidelines, the Maastricht 2-2000 Consensus Report (4), introduced the concept of a 'treatment package', in which the second antibiotic of the first-line therapy should be amoxicillin and not metronidazole, which must be reserved in case a second antibiotic of the first-line therapy should be amoxicillin and not metronidazole, which must be reserved in case a second antibiotic of the first-line therapy is needed.

One limitation of these treatments is compliance. In a study using Medication Event Monitoring System containers, lack of compliance occurred in more than 10% of the patients, essentially due to adverse events (5). A more important limitation is the resistance of *H pylori* to antimicrobials, especially to clarithromycin.

In the present paper, we will consider *H pylori* resistance to clarithromycin and to other antibiotics, and for each antibiotic, the global resistance rate, the impact on treatment success and new information will be presented.

**RESISTANCE TO CLARITHROMYCIN**

A survey performed in different countries across Europe, using the same methodology in each country (epsilometer [E]-test), showed a global clarithromycin resistance rate of 9.8%. There was a large variation between northern Europe (4.2%) and southern Europe (18.4%), with central and eastern Europe being somewhat in the middle range (9.3%) (6) (Figure 1). This can be attributed to differences in antimicrobial use. As in the case of *Streptococcus pyogenes* (7), *H pylori* resistance to macrolides seems to be dependent on the selective pressure resulting from a high consumption of these drugs (8). In Europe, a survey of children indicated a clarithromycin resistance rate of 20% (S Koletzko, personal communication).

This presents a potential problem because, when these children become adults, they will transmit strains that are already clarithromycin-resistant to their descendants and the global resistance rate may become more important.

In the United States, clarithromycin resistance rates of 12% (9) and 15% (10) have been reported, while in Canada it is 12% (11). Recent data from Asia indicate a clarithromycin resistance rate of 4%, 6% and 7.6% in Korea, Taiwan and...
Japan, respectively, while it was 14.4% in Australia. Macrolide resistance is important to consider because of its clinical relevance. Most of the studies comparing the cure rates in patients infected by susceptible and resistant *H pylori* strains show a huge difference, ranging from 90% success to less than 20% to 30%, respectively (10,12,13).

The mechanism of macrolide resistance is straightforward (14). Point mutations occur in two positions on the 23S rDNA (15), which change the spatial structure of the ribosome and inhibit macrolide binding (16). Mutations in other sites probably lead to nonviable bacteria or alter the fitness of the bacteria and, therefore, are not found.

In addition to the standard phenotypic methods of susceptibility testing, several molecular methods have been proposed (Table 1).

### What’s new?
A standard protocol to test clarithromycin susceptibility was proposed by the NCCLS several years ago (25). This protocol gives excellent results but, because agar dilution is necessary, it is difficult to implement in routine practice. For this reason, an attempt was made to validate a disk diffusion method in France. The results showed that the best method to discriminate between susceptible and resistant strains of *H pylori* is to use an erythromycin disk on a Mueller Hinton agar plate, with a critical diameter of less than 17 mm for resistant strains (26).

Polymerase chain reaction (PCR)-restriction fragment length polymorphism remains the most accessible molecular method for routine laboratories. Unfortunately, no enzyme had been described to detect the A2142C mutation. The enzyme BceAI, which leads to three bands instead of two being produced when the mutation is present, was recently proposed (27).

New molecular methods have been developed. Two studies (28,29) have reported the possibility of detecting *H pylori* directly in gastric biopsies, using a real-time PCR based on the Fluorescence Resonance Energy Transfer (FRET) methodology, and of testing macrolide susceptibility by melting curve analysis (MCA) in a LightCycler apparatus (Roche Diagnostics, France) However, only a small number of biopsies with resistant strains were tested in one study, and the other study did not test for the A2142C transversion. The new FRET-MCA protocol developed in our laboratory has the capacity to detect all known mutations within two hours after receiving the biopsy, one hour being used for DNA extraction, and one hour for performing the test. We tested this protocol on 200 biopsy specimens obtained from patients who had failed eradication treatment with a clarithromycin-based triple therapy. The sensitivity and specificity compared with agar dilution were 98.4% and 94.1%, respectively (30). Interestingly, there were 42 cases with a mixture of wild type and resistant mutants, which were detected more easily than with the phenotypic method.

The mutations that occur spontaneously are then selected by the drug. When resistant mutants constitute an essential part of the *H pylori* population, given that the mutations do not have any impact on the bacterial fitness, the number of mutants in the population do not decrease with time (31).

## RESISTANCE TO OTHER ANTIBIOTICS

### Amoxicillin
Amoxicillin is the second antibiotic commonly used to treat *H pylori* infection. Fortunately, resistance to this drug appears to be very limited. Indeed, Dore et al (32) reported the existence of *H pylori* strains tolerant to amoxicillin (ie, the ratio of the minimal bactericidal concentration to the minimal inhibitory concentration [MIC] is greater than 32 mg/L). This tolerance was not always observed after freezing of the strains. The authors hypothesized that it was due to a lack of a certain penicillin binding protein (PBP), named PBP-4 (33). The clinical significance of this phenomenon is not clear.

Another resistance mechanism was described in a strain isolated in the Netherlands from a patient who had received multiple courses of amoxicillin for respiratory tract infections. The moderate level of resistance (8 mg/L) was transferable (34).

### What’s new?
By sequencing, Gerrits et al (35) have shown that a single point mutation on the *ppbA* gene concerning a serine 414-arginine substitution was able to increase the MIC of susceptible strains to 1 mg/L after transformation.

Another study using an *H pylori* strain selected by culturing an amoxicillin-susceptible strain in increasingly higher concentrations of amoxicillin resulted in a MIC increase from 0.02 mg/L to 15 mg/L. The resistant strain showed mutations on *ppbI* involving four amino acids. However, the transformation of a susceptible strain with the mutated *ppbI* gene rendered these strains only moderately resistant, suggesting that mutations in other genes may be involved (36).

In a similar experiment, in which the strain MIC increased from 0.03 mg/L to 4.8 mg/L, the resistant strain exhibited a sig-

### Figure 1) Resistance of Helicobacter pylori to clarithromycin in Europe
![Graph showing resistance levels in different regions.](image)

### Table 1: Molecular methods for *Helicobacter pylori* testing of clarithromycin resistance

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<tr>
<th>Method</th>
<th>PM1</th>
<th>PM2</th>
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<th>PM4</th>
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<tr>
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<td>9.8</td>
<td>4.2</td>
<td>9.3</td>
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<td>DNA enzyme immunoassay</td>
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<td>Preferential homoduplex formation assay</td>
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<td>Line probe assay</td>
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<td>Mismatch polymerase chain reaction</td>
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TABLE 1

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<td>Based on amplification of 23S rRNA gene</td>
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<td>Sequencing</td>
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<td>Restriction fragment length polymorphism (15,16)</td>
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<td>Oligonucleotide ligation assay (17)</td>
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significant decrease in PBPl binding, and a decrease in the uptake of the labelled amoxicillin (37).

Resistance to amoxicillin is still seldom found and, so, its impact on the clinical outcome of treatment is still not known. However, it is expected that an increase in the future due to selective pressure will be seen.

No molecular method is currently available to detect this resistance. The best method consists of measuring the strain MIC, and this is achieved using the E-test.

Metronidazole

In recent guidelines, metronidazole was recommended in second-line therapies, and especially in quadruple therapy.

The prevalence of H pylori resistance to metronidazole is quite high. In Europe, the previously reported study indicated a global resistance rate of 33% with a higher rate in southern Europe (40.8%) (6). A similar resistance rate is found in the United States (39%) (9), Taiwan (32%) and Australia (32%), but not in Japan (4.1%), because this drug is seldom used there.

The impact of metronidazole resistance on the clinical outcome of H pylori eradication is relatively modest: the eradication rate decreases by 20% when the strain is resistant rather than susceptible (38).

The mechanism of metronidazole resistance is not yet fully understood. Metronidazole must be reduced to exert its damage on bacterial DNA. The main enzyme involved is an oxygen-insensitive nitroreductase rdxA as reported by Goodwin et al (39). Mutation in the rdxA gene can alter its nitroreductase activity and render the organism resistant. Several mutations may be involved and, furthermore, all rdxA mutations are not associated with a resistant phenotype. In addition, the RdxA protein may be produced but may not express its activity. Another gene, frxA, can modulate the expression of rdxA, but this is a topic of controversy as was highlighted in a recent review (40). Some authors believe that frxA mutations may also lead to a resistant phenotype (41), while others see frxA only as a molecule which enhances the level of resistance of rdxA mutants (42).

What’s new? The difficulty in performing H pylori susceptibility testing for metronidazole has been reported many times. In a European study comparing the E-test with the agar dilution method used in four laboratories, major errors for categorizing the susceptibility to metronidazole were found in 32.5% of the cases, indicating an unacceptable intertest variability (43).

The optimal method may, in fact, be to look for the RdxA protein by immunoblot as proposed by Latham et al (44). They obtained a positive result in 100% of 17 strains categorized as positive, but only in 7.5% categorized as negative.

Tetracycline

Tetracycline is also used in second-line treatments, and especially in the quadruple therapy.

The first resistant strain was isolated in Australia, but the mechanism was not known at that time (45). Subsequently, other reports have been published, but this resistance remains rare except in Korea, where a 5.3% (46) rate was reported and resistance could be transferred (47). However, the impact on clinical outcome has not yet been documented.

What’s new? Importantly, the mechanism has been described independently by two groups. Trieber and Taylor (48), studying the first reported Australian resistant strain, found a triple mutation AGA965-967TTC in the 16S rRNA gene, in a region adjacent to the tetracycline binding site. Gerrits et al (49), using another resistant strain, observed that the same triple mutation was present, indicating a high probability of a causal association.

Rifamycins

Among the rifamycins, only rifabutin has been proposed as a rescue therapy. No resistance has been reported in vivo, but resistance can be selected in vitro. Heep et al (50) reported that resistant cases were due to point mutations in the 69 base pair region of the rpoB gene; this gene codes for the beta subunit of the RNA polymerase that is the target of rifamycins.

Quinolones

Recently, several studies have shown a good efficacy for cefuroxacin. The rate of resistance is still low (3% in France), but may be more important in countries with a high consumption of quinolones, such as Portugal. In a study dating back to 1995, it was shown that resistance, which occurs easily, is associated with three point mutations in the gyrA gene (51).

IMPLICATIONS

It is interesting to note that all of the known mechanisms of resistance have now been described for antibiotics commonly used for H pylori eradication. They are all related to point mutations of genes present on the bacterial chromosome (Table 2), making genetic transfer unlikely, except possibly by transformation. One consequence is the slow increase in resistance because it only concerns the descendants of the mutated bacteria and is not a horizontal transfer. Nevertheless, when resistance to macrolides reaches 20%, as it does in France, the strategy of empiric use of clarithromycin must be challenged.

We now have a method that allows a 2 h diagnosis of H pylori infection and of its susceptibility to macrolides, making it worthwhile to perform susceptibility testing to provide a tailored choice of treatment, rather than an empirical clarithromycin-based treatment.

For the detection of metronidazole resistance, a new testing method based on the immunoblot may become the method of choice because molecular methods cannot be applied and standard methods are notoriously unreliable.

In summary, progress in eradication therapy will be a result of the progress in susceptibility testing, and we are now close to revising the guidelines to propose susceptibility testing before the first treatment is attempted, rather than waiting for a failure of cure.


