Antibiotic resistance in *Helicobacter pylori* infection

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Resistance to antibiotics is considered as the primary reason for failure of eradication therapies. Resistance to clarithromycin is due to a decrease in binding to the ribosomes associated with a point mutation on the 23S rRNA. Its rate in Europe varies from 0–15%, with 5% in the UK. The resistance influences dramatically the success of the treatments. Resistance to metronidazole is due to a lack of reduction of this compound whose genetic basis is still unknown. The resistance rate in Europe varies from 10–50%, with 25% in the UK. It influences the success of treatments to a lesser extent than clarithromycin resistance.

The initial eradication treatment can be prescribed without testing for susceptibility and must include a combination of two antibiotics, while stressing the importance of compliance to the patient. In case of failure, susceptibility testing must be performed.

Few data are currently available on alternative therapeutic strategies when *H. pylori* is resistant to both clarithromycin and metronidazole.

Despite the fact that the optimal treatment for *Helicobacter pylori* infection has not been found, there are several combinations of drugs which can cure the infection in up to 80–90% of cases. There are several possible explanations for failure, such as a lack of compliance from the patient, but the main reason to date seems to be the occurrence of strains which are resistant to the antibiotics used. Among the compounds recommended for eradication of *H. pylori*, resistance occurs frequently with macrolides and nitroimidazoles but never with amoxicillin and tetracycline, although some anecdotal reports exist for the latter two (Table 1). This review will present current data concerning the mechanisms and the epidemiology of the resistance and consider ways to deal with this problem in clinical practice.

**Resistance mechanisms**

**Resistance to macrolides**

Macrolides are a group of antibiotics with a lactone ring of which erythromycin is the leader. These antibiotics are administered orally,
Helicobacter infection

Table 1 MIC of different antibiotics on susceptible and resistant strains of *H. pylori* (mg/l)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible strains</th>
<th>Resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>0.015 - 0.12</td>
<td>4 - 64</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.015 - 0.06</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.25 - 2</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.06 - 1</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.12 - 0.5</td>
<td></td>
</tr>
</tbody>
</table>

they diffuse well in tissue, have a narrow spectrum and limited side effects. They are used to treat *H. pylori* infection in association with a second antimicrobial agent, either amoxicillin or a nitroimidazole compound, and an antisecretory drug. The most attractive compound to date is clarithromycin because it has the best efficacy *in vitro*. It is moderately affected by a decrease in pH, and good concentrations can be obtained in gastric mucosa.

Once macrolides have penetrated into the bacterial cell, they bind tightly to ribosomes where protein synthesis takes place. The exact site of action is the peptidyl transferase loop of domain V of the 23S ribosomal RNA. In the event of resistance (MIC > 2 mg/l), there is a decreased affinity between ribosomes and clarithromycin. This property has been found to be associated with a point mutation in the 23S rRNA gene in position 2143 (formerly 2058 in the *Escherichia coli* nomenclature) and 2144 (formerly 2059). This result has been confirmed by others including studies on European strains. The mutation is essentially a transition A→G but may be a transversion A→C in position 2143 in some cases. Detection of this mutation is possible after PCR using adequate primers amplifying part of the 23S rRNA gene followed by sequencing or restriction with the enzymes *Bsal* (A2143G) and *Bbsl* (A2144G) but not A2143C. Recently, methods based on hybridization of the amplified products have been proposed, such as the PCR-oligonucleotide ligation assay.

Resistance to nitroimidazoles

Metronidazole and tinidazole are the compounds in this class used to treat *H. pylori* infection. Cross resistance between these two drugs exists. The essential differences between them relate to their pharmacodynamics and safety profiles.

To be active, metronidazole must penetrate into the bacteria. Subsequently, the NO₂ group of the nitroimidazole compound is reduced to form a hydroxylamine derivative. The reduced product causes damage to the DNA thereby causing cell death.
Smith and Edwards studied drug uptake and the rate of cell killing of metronidazole against *H. pylori* and found it dependent upon the relative oxygen tension of the environment and the cell density, both of which determine the redox conditions of the media. These results are in agreement with the work of Cederbrant *et al* who showed that *in vitro* metronidazole resistance could disappear when the strains were incubated anaerobically for a few hours before growth. The low redox potential thus achieved led to an increased reduction of metronidazole, the active compound generated.

The mechanism which renders the metronidazole resistant strains of *H. pylori* susceptible after a short anaerobic incubation is not yet known. The activation of an anaerobic metabolic pathway which does not function under microaerobic conditions could be the cause and would also explain the apparent non-stable resistance, since the atmosphere of incubation is not fully controlled during subculture of the strains.

The genetic mechanism which underlines the resistance is not yet known and, therefore, a molecular detection is not possible.

**Resistance to fluoroquinolones**

While fluoroquinolones are not routinely included in current regimens used to treat *H. pylori* infection, ciprofloxacin is sometimes used in the event of resistance to previously employed compounds.

Fluoroquinolones interfere with DNA gyrase, an enzyme acting on the supercoiling of DNA during cell replication. In one study, resistance was found associated with four groups of point mutations in the *gyrA* gene. A cross resistance also exists for this group of compounds.

**Resistance to tetracyclines**

While currently not used extensively, tetracyclines are a component of the bismuth-based triple therapy regimen which was recommended in 1990 to treat *H. pylori* infection. Their site of action is the ribosome.

It is thought that *H. pylori* does not acquire resistance to tetracyclines. However, this conception must be reconsidered following a recent description of a resistant strain in Australia. More information is needed, particularly with regards to the mechanism of action.

**Resistance to β-lactams**

Amoxicillin is the β-lactam most commonly used in the regimens aiming to eradicate *H. pylori*. One might ask why *H. pylori* has not acquired resistance to this drug while, during more than 20 years, tons of amoxicillin have been used.
The main mechanism of resistance in this group is linked to the production of β-lactamase, a resistance commonly found in Gram-negative bacilli and staphylococci. It seems that the *H. pylori* restriction enzymes do not allow the introduction of exogenous DNA from phylogenetically distant bacteria. Moreover, the possible contacts between *H. pylori* and these bacteria are most likely to be limited unless one believes that *H. pylori* can multiply in the intestine. Nevertheless, β-lactamase genes can theoretically be transferred to *H. pylori*.

A second mechanism of resistance is a modification of the target, *i.e.* the penicillin binding proteins. Several of them may be present in *H. pylori*. It is possible that, if a resistance occurs in the future, it will be by this mechanism, as was the case with *Streptococcus pneumoniae* in recent years. In this case, the levels of resistance are usually relatively low.

MICs of amoxicillin against *H. pylori* are usually low (0.03 mg/l). It is, however, possible to find strains with MICs 10 times higher (0.25–0.50 mg/l) which warrant further study. The small number found in clinical trials does not allow an association between these strains and treatment failure to be detected. Furthermore, Dore *et al.* recently reported tolerance to amoxicillin, however, this phenomenon seems difficult to maintain in subcultures. For these reasons, susceptibility of *H. pylori* to amoxicillin must be monitored.

### Epidemiology of resistance

#### Resistance to macrolides

The frequency of resistance to macrolides varies from country to country and seems to parallel the use of this therapeutic class to treat other infections, mainly respiratory tract infections in the past. There is a north–south gradient. In Scandinavia where macrolides have rarely been used, the level of *H. pylori* resistance remains very low while the rate is 10–15% in Southern Europe.

There is a tendency towards an increase in resistance in countries where macrolides have recently been introduced. In Belgium, for example, the resistance rate increased from 2.2% in 1990 to 11.1% in 1996. It is, therefore, mandatory to have a surveillance of *H. pylori* resistance to macrolides.

#### Resistance to nitroimidazoles

A marked difference has been found between the rate of resistance to nitroimidazoles in developed and developing countries. This difference
Antibiotic resistance

may be linked to the high level of general use of metronidazole in
developing countries, because these countries are mostly tropical countries
and this inexpensive drug is commonly used to treat parasitic infections
such as amoebiasis. Resistance rates can be as high as 80–90% in
developing countries, as reported in Africa (Burkina Faso, Zaire).

In developed countries, the rate of resistance ranged from 10–50% in a
European multicenter study in 1991\textsuperscript{18}. The cause of this resistance may
also be linked to the use of these compounds for genital infections,
especially trichomoniasis and, therefore, strains isolated from women are
more likely to be resistant than strains isolated from men\textsuperscript{18}. Another
possible cause may be the use of these compounds to treat dental
infections.

There is a trend towards an increased rate of resistance in developed
countries. This may be due to an increased use of these compounds to
treat \textit{H. pylori} infection, because resistant organisms can easily emerge.
However, this trend may not be of the magnitude suspected. Indeed, there
are problems with regard to the technique used to detect this resistance.
In our experience only MICs determined by agar dilution, and not Etest,
correlate with the clinical outcome in clinical trials\textsuperscript{19}.

\textbf{Resistance to fluoroquinolones}

There have been very few surveys concerning resistance to fluoro-
quinolones. This resistance is usually reported to be less than 1%. Up until
now, fluoroquinolones have been used mostly to treat severe infections in
hospital settings and not in the community as is the case for macrolides.
When they are used, the treatment lasts for a very short period. Hence, we
can conclude that exposure to fluoroquinolones has been limited.

\textbf{Problems observed during the initial treatment for \textit{H. pylori}}

These problems concern the possible existence of ‘primary’ resistance to
the compound administered, as well as the possible occurrence of a
secondary resistance to the treatment prescribed, both of which can lead
to a treatment failure.

\textit{‘Primary’ resistance}

This is always the consequence of previous treatment with the antibiotic
considered, even if the patient has no recollection of previously taking
the drug.
The Maastricht consensus report states that susceptibility testing is not mandatory before prescribing the initial treatment\textsuperscript{20}. The treatment will then be 'probabilistic' and, therefore, must be based on epidemiological data of resistance, either national, or better yet regional. It is currently accepted that, with a resistance rate of \textit{H. pylori} less than 15\% to clarithromycin and less than 30\% to metronidazole, it is still reasonable to use these drugs.

It must be kept in mind that when there is resistance to one compound, a certain efficacy will come from the other compound. For instance, amoxicillin as the only antibiotic associated with an antisecretory drug which can lead to a 50\% success rate and the rate increases to 70\% for clarithromycin. The expected success rates according to the rate of resistance to clarithromycin for the omeprazole-amoxicillin-clarithromycin treatment are presented in Figure 1 and according to the rate of resistance to metronidazole for the omeprazole-clarithromycin-metronidazole treatment are presented in Figure 2.
Antibiotic resistance to metronidazole for the omeprazole-clarithromycin-metronidazole treatment are presented in Figure 2. These expected rates are based on the results of different clinical trials performed in Europe, i.e. ACT 10\textsuperscript{21} and MACH 2\textsuperscript{22}.

**Secondary resistance**

Since the resistances concerned are acquired by mutation, it is important that the antibiotics always be present in sufficient quantities at the site of the infection to avoid the emergence of resistance.

It is mandatory always to prescribe two antibiotics and to explain carefully to the patient that the compounds must be taken together and without interruption until the end of the 7 day period. Compliance to treatment is a key factor in preventing the occurrence of resistant strains.

**Problems occurring after failure of initial therapy**

Failure or eradication will be noticed either by a relapse of symptoms or by a positive test for \textit{H. pylori} during post-therapy follow-up. Except under special conditions, i.e. gastric ulcer and gastric lymphoma, follow-up is performed by a $[^{13}\text{C}]-\text{urea breath test}$. This test is very specific and its sensitivity post-treatment is higher than 90%.

**Susceptibility testing of \textit{H. pylori} to antibiotics**

In the event of a positive result, testing the susceptibility of \textit{H. pylori} to antibiotics before prescribing a new eradication treatment is recommended. This is undoubtedly the main reason motivating \textit{H. pylori} culture. \textit{H. pylori} is a fragile and slow growing bacterium so it is advisable to refer to specialized laboratories for its culture. With regards to susceptibility testing, all techniques can be used for macrolides while the breakpoint method (8 mg/l) can be recommended for metronidazole and the Etest for amoxicillin.

A delay of several days is needed in all cases before obtaining results.

**Management after failure of a first eradication therapy**

It is not possible to find answers to this question in the literature, because no trial has yet been performed. However, because cases of failure to eradicate \textit{H. pylori} are becoming more frequent, such studies...
are urgently required. The approach outlined below is only subjective but is facilitated by knowledge of the susceptibility of *H. pylori*.

If the initial antimicrobials given were clarithromycin and amoxicillin, in the event of clarithromycin resistance, metronidazole should be given. If the initial antimicrobials given were clarithromycin and metronidazole, in the event of clarithromycin resistance, amoxicillin should be given; and if there is a metronidazole resistance, amoxicillin can also be used.

However, the situation becomes more complicated if the bacterium is resistant to both clarithromycin and metronidazole. The results of one unpublished trial indicate that the combination of amoxicillin-metronidazole is more effective when the dose of metronidazole is increased to $3 \times 500$ mg/day. Furthermore, it is advisable to increase the duration of treatment from 7 to 10 days.

Other combinations, *i.e.* amoxicillin-tetracycline and amoxicillin-ciprofloxacin, should also be considered. Quadruple therapy may also be a good alternative. It consists of adding a proton pump inhibitor to standard triple therapy: bismuth salts-tetracycline-metronidazole. A new compound, ranitidine bismuth citrate (RBC), combines an antisecretory drug, ranitidine, and an antibacterial drug, bismuth salts, in one molecule. In association with clarithromycin for 2 weeks, RBC achieves eradication rates similar to proton pump inhibitor-based eradication regimens. When combined with two antibiotics, a quadruple therapy is then achieved and this combination given for 7 days may lead to high eradication rates.

In case of failure without resistance, the same combination can be given a second time but for 2 weeks instead of 1 week, while stressing the importance of compliance.

**Conclusion**

In conclusion, *H. pylori* resistance to antibiotics is the first cause of failure of current eradication regimens. We can expect progress in the field of susceptibility testing, *e.g.* standardisation of the procedures and development of rapid tests.

The main progress will, however, come from using simpler regimens to which *H. pylori* will not develop resistance. Nitazoxanide, a nitrothiazole compound similar to metronidazole which has this characteristic, is currently under study. Knowledge of the entire sequence of the *H. pylori* genome should permit the development of drugs which would target specifically vital functions of *H. pylori* in the near future.
References

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