Antimicrobial resistance in *Helicobacter pylori* : a global overview

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Abstract

Helicobacter pylori **resistance to antimicrobial agents is of particular concern because it is a major determinant in the failure of eradication regimens. Antimicrobial drug resistance has been reported to occur for nitroimidazoles, macrolides, fluoroquinolones, rifampin and tetracyclines. Resistance to nitroimidazoles is the most common, in the range of 30-40% on the average in Europe while the overall prevalence rate of resistance to macrolides is lower, probably ranging between 2-10% in most countries. Development of secondary (acquired) resistance to nitroimidazoles and to the macrolides usually occurs as a rule (> 70-100%) in case of failed eradication therapy. Data available from several centres seems however to indicate that a significant shift towards increasing resistance to metronidazole and to the macrolides might have possibly occurred in many countries over the last years.**

Resistances to both metronidazole and to clarithromycin are the most significant ones because they influence the success of the treatments although this seems to be less marked and more dependent on the treatment regimens considered in the case of metronidazole resistance than in the setting of clarithromycin resistance. These differences may in part relate to methodological variations and to the inherent difficulties in assessing the susceptibility of *H. pylori* to metronidazole. It is possible that different resistance cut-off might also have to be considered for metronidazole depending on the treatment regimens administered.

The mechanisms of resistance have been well defined for the macrolides and are beginning to be unraveled for the nitroimidazoles. In all cases, resistance of *H. pylori* to antimicrobial agent seems to be due to the development of single mutational events in chromosomal genes rather than to the acquisition of exogeneous resistance genes. Owing to the restricted ability of microbiology laboratories with expertise in *H. pylori* culture and the lack of standardised methodology for susceptibility testing, *H. pylori* culture is not often performed routinely. It should however be considered after documented treatment failure or in patients from a geographic area or of an ethnic origin with higher likelihood of antimicrobial drug resistance. Likewise it is deemed very important to institute national and regional surveillance programs to follow the evolution of *H. pylori* resistance and to better adapt treatment regimens to changes in resistance patterns. (Acta gastroenterol. belg., **1998**, 61, **357-366**).

Introduction

Over recent years considerable progresses have been accomplished in the field of *H. pylori* therapy, and there are several current drug regimens which can cure the infection in up to 80-90% of the cases (1). Besides the lack of compliance of the patient to the treatment, antimicrobial drug resistance has appeared as one of the principal reason for treatment failure (2,3,4). Because of the high prevalence of *H. pylori* infection worldwide and the extending number of indications for therapy of this infection (5), it is logical to believe that the issue of *H. pylori* resistance to antibiotics may become even more important in the future. Indeed, several reports have suggested that antimicrobial resistance of *H. pylori* might well have increased over the last years as a consequence of previous treatment (6,7,8,9).

The aim of this paper is to review current available data concerning the mechanisms, the epidemiology and the clinical relevance of drug resistance in *H. pylori.* It also gives some recommendations concerning the ways to deal with this problem in clinical practice.

Definition of resistance of H. pylori to antibiotics

Antimicrobial resistance to a specific agent is usually defined according to the concentration of active drug which reaches the site of infection where the organism is present, in relation to the concentration of drug that is necessary to inhibit its growth (the minimal inhibitory concentration [MIC]). A strain is categorized as resistant either when it can tolerate significantly higher antimicrobial concentrations than the concentration inhibiting the growth of other strains of the same species (bacteriological resistance) or when it can tolerate drug concentrations higher than those achieved *in vivo* at the site of the infection (pharmacological resistance). From a practical point, because of the inherent difficulty of determining specific breakpoint values for each tissue, only the serum drug concentrations are usually considered. These classical definitions of resistance might however not be appropriate in the case of *H. pylori* since blood and gastric mucosal concentrations are usually very different from each other for most antibiotics, and also because these can vary widely at different levels of the mucosa or in different areas of the stomach. Moreover, the MIC values for some antibiotics are very dependent on the pH of the gastric milieu, which may also be found very variable from one subject to another.

As a consequence, a clinical approach is the most valuable for defining resistance. Following this approach, a strain is defined as resistant when the likelihood of eradication by a given treatment is very low. Hence, breakpoint values for resistance of *H. pylori* to antimicrobial agents should be determined by establishing clinico-bacteriological correlations between the MIC values determined using a reference method before treatment and the clinical efficacy assessed in terms of bacterial eradication. Unfortunately, such data are not often available because the availability of effective regimens is relatively recent and also because

few of the published clinical trials have included bacteriological data.

Resistance mechanisms

Primary and secondary (acquired) resistances of *H. pylori* have been reported for several groups of antibiotics including the macrolides, the nitroimidazoles, the fluoroquinolones, rifamycins and the tetracyclines (3,4). From a clinical point, only resistance to the nitroimidazoles and to the macrolides is important since the other compounds are not included in current therapeutic regimens of *H. pylori* infection. A summary of the different classes of antimicrobial agents selecting resistance in *H. pylori* is shown in Table I.

Table I. — **Acquired resistance of *H. pylori* to antimicrobial and non-antimicrobial agents**

Agents not inducing resistance	Agents inducing resistance
Amoxicillin and other beta-lactam agents	Fluroquinolones (ofloxacine, ciprofloxacine, norfloxacine)
Nitrofurantoine, Furazolidone	5-Nitroimidazole drugs (metronidazole, tinidazole)
Bismuth salts	Macrolides (clarithromycin, azithromycin, erythromycin) and related lincosamides (clindamycin)
Proton pump inhibitors	Tetracycline Rifampin

After references 2, 3, 4.

Nitroimidazoles

Metronidazole and tinidazole are the two major compounds of this class used for the treatment of *H. pylori* infection. Cross-resistance does occur between these two drugs. To be active, metronidazole must penetrate into the bacteria to be subsequently reduced on its NO_2 group to form a hydroxylamine metabolite. This active reduced compound will than break down bacterial DNA and will hence cause cell death (10). Cederbrant *et al.* have shown that exposure of metronidazole-resistant *H. pylori* isolates to an anaerobic environment for a few hours before growth caused the activation of metronidazole and the loss of resistance (11). They proposed that metronidazole resistance would result from the inability of some *H. pylori* strains to achieve in microaerobic conditions a sufficiently low redox potential necessary to reduce metronidazole in its active metabolite. Smith and Edwards studied the metronidazole uptake and cell killing rate against *H. pylori* and found it to be dependent upon the relative oxygen tension and the cell density, both of which determine the redox potential of the media (12). In a subsequent study, the same authors showed that metronidazole-resistant *H. pylori* strains had a decreased oxido-reduction activity (10-fold decrease in NADH oxidase activity in resistant — as compared to metronidazole-susceptible strains) and that they were not able to remove intracellular oxygen from the site of metronidazole thereby preventing the reduction of metronidazole (13). Very recently, Hoffman *et al.* have for the first time identified and sequenced a gene (14), called *rdx*A which encodes an oxygen insensitive NADPH nitroreductase that can reduce metronidazole *in vitro*. The metronidazole-resistant and sensitive *H. pylori* strains differed from each other only by a few point mutations in the *rdx*A gene sequence. Construction of isogenic mutants by insertion of a chloramphenicol-resistance cassette in the *rdx*A gene, resulted in a metronidazole-resistance phenotype equivalent to that found in metronidazole-resistant clinical isolates. Conversely, introduction of a functional *rdx*A allele into a metronidazole-resistant strain on a shuttle vector plasmid rendered that strain sensitive to metronidazole. This finding appears very promising since it may lead to the development of molecular tests in a near future. Such tests could indeed be well indicated for the detection of metronidazole resistance since conventional testing methods have often appeared as poorly reliable when testing *H. pylori* against this class of agents. The importance of this mechanism among resistant strains is however not yet known and it is still possible that other mechanisms of resistance to metronidazole may also exist.

Macrolides

The macrolides presently constitute a group of major importance for the treatment of *H. pylori* infection because of their excellent *in vitro* activity against *H. pylori*, their favorable pharmacokinetics properties and safety profiles. Clarithromycin has emerged to date as the most attractive compound among this group since it displays the best activity *in vitro* and also because it is less affected than other macrolides by a decrease in pH. Macrolides act by binding to ribosomes, and more precisely the peptidyl transferase loop of domain V of the 23S ribosomal RNA (15). They eventually inhibit protein synthesis by inducing dissociation of peptidyl tRNA from the ribosome during the elongation reaction. Macrolide resistance in bacteria is usually due to several mechanisms including lack of macrolide binding to the ribosome target, macrolide inactivation by enzymes, impermeability of the bacterial membrane and active drug efflux (16).

Several studies have indicated that clarithromycin resistance was associated with a point mutation located in the 23S rRNA gene in position 2143 and 2144 (17,18). The mutation is essentially a A→G transition mutation in 2143 or 2144 but in a few cases it may be a A→C transversion mutation in 2143 (19,20). Such point mutations result in a decreased affinity between the ribosomes and clarithromycin and in a marked increase in the MIC values (> 2 µg/ml) (20). A2143G mutations are usually associated with higher resistance levels (MIC ≥ 64 µg/ml) than those of the A→G type

located at position 2144 (MIC $\leq$ 32 µg/ml) (21). Resistance to clarithromycin is crossed to all other macrolides and it stably persists over time *in vivo* and also after multiple *in vitro* subcultivations (22). Detection of this mutation is possible by PCR with adequate primers amplifying part of the 23S rRNA gene subsequently followed by sequencing (20,23) or after restriction with the *Bsal* (A2143G) or *Bsbl* (A2144G) enzymes (24). The A2143C mutation can however not be detected by the PCR-RFLP technique since this mutation does not lead create an additional restriction for these restriction enzymes.

Recently, rapid methods based on the detection of the amplified products by hybridization with a specific oligonucleotide probe have been proposed (21,25). The latter technique looks very promising since it is prone to automation and also because it could be applied directly to biopsy specimens in parallel to a method detecting specific *H. pylori* genes.

Fluoroquinolones

Although fluoroquinolones are usually not included in therapeutic schemes for the eradication of *H. pylori*, ciprofloxacin has been used occasionally in the setting of resistance to previously used compounds. Fluoroquinolones specifically interfere with DNA gyrase, an enzyme acting on the supercoiling of DNA during cell replication. In one study, resistance to fluoroquinolones has been found to be associated with four groups of point mutations in a specific part of the *gyrA* gene (26). These mutations were systematically associated with an increase in the ciprofloxacin MICs from $\leq$ 0.25 µg/ml to 8 µg/ml and with cross-resistance to all other fluoroquinolones compounds (26). Interestingly, resistance could be transmitted *in vitro* by transformation of amplified DNA fragments from ciprofloxacin-resistant strains into susceptible isolates.

Tetracycline

While currently not used extensively, tetracyclines have been included as part of the bismuth-triple therapy which was recommended in the early 90's as the mainstay regimen to treat *H. pylori* infection. These agents inhibit the protein synthesis by interacting with the ribosome. Resistance of *H. pylori* to tetracyclines, once considered as nonexistent has rarely been reported in *in vitro* surveys (27,28). High level resistance to tetracycline (MIC $>$ 256 µg/ml) has also recently been detected in one *H. pylori* strain in Australia from a patient in whom eradication had failed with triple therapy (29). The underlying mechanism of resistance however still remains to be determined.

Beta-lactams

It is puzzling that acquired resistance to beta-lactam has not yet been observed in *H. pylori* while amoxicillin and other beta-lactams have been so extensively used in the community for more than 30 years. Production of beta-lactamase usually accounts as the most frequent mechanism of resistance to this class of antibiotic. Such resistance has largely spread in various groups of bacteria but it has not been formally detected in clinical *H. pylori* isolates. This may possibly relate to the fact that contacts and opportunities for genetic exchanges between *H. pylori* and other bacterial species are likely to be limited in the gastric mucosa environment. Nevertheless, beta-lactamase genes have been transferred *in vitro* to *H. pylori* to study virulence factors by shuttle mutagenesis (30).

A second mechanism of bacterial resistance to beta-lactams is a modification of the bacterial cell wall target (i.e. the penicillin protein bindings [PBPs]). Successive mutations or repeated acquisition of foreign DNA by genetic transformation have resulted in modifications of the PBPs with a subsequent decrease in their affinity for antibiotics acting at the cell-wall level. Such mechanism, which accounts for a relative low-level resistance and a stepwise increase over time in the MIC values, has recently emerged in several bacterial species (i.e. *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*). Owing to its high natural DNA transformation ability, beta-lactam resistance could perhaps also occur in *H. pylori* in the future by this mechanism. One group recently claimed having isolated several amoxicillin-resistant (MIC $>$ 256 mg/l) or tolerant strains (i.e. strains inhibited but not killed at high antibiotic concentrations) from patients in Italy and in the US (31,32). However this phenomenon seemed to be difficult to maintain in subcultures and tended to disappear following storage of the strains at low temperature (32). Until a precise mechanism of resistance is reported, the real significance of this finding remains unknown. MIC values of amoxicillin against *H. pylori* are usually uniformly low (0.015-0.03 µg/ml). It is however possible to found very occasionally strains with MICs 10 or more times higher (0.25 to 0.5 µg/ml) (33). While it has not been possible to establish any clinical association between the presence of these "less susceptible" strains and treatment failure, it could be interesting to characterize them further from a bacteriological point. In any cases, these various observations underscore the importance of keeping monitoring the susceptibility of *H. pylori* to amoxicillin in order to detect possible new cases of resistance.

Other antibiotics

Resistance to rifampin and to related derivatives can be acquired but the rate is very low. The mechanism has not been studied and it is probably of little importance since this class of antimicrobial agents has no indication for the treatment of *H. pylori* infection. Resistances to compounds, which have no clinical importance for *H. pylori* (chloramphenicol, kanamycin, streptomycin), have been transmitted by transformation *in vitro*.

Table II. — **Primary resistance rates of *H. pylori* to antibiotics in different geographic areas (1990-1997)**

Agents	Average resistance rate	Ranges	Geographic area
Metronidazole (tinidazole)	30-40%	< 10→90%	Europe < 10-50% United States 20-50% South America 30→80% Africa 70-90% Asia, Pacific 20-70%
Clarithromycin (azithromycin, erythromycin roxithromycin)	2-10%	< 1-17%	Europe ≤ 5% (except Belgium, France, Spain, Poland : ≥10%) United States, Canada : 2-13% Asia, Pacific : 2-10%
Ciprofloxacin (ofloxacin, norfloxacin)	≤ 1%	1-5	Europe, United States
Tetracycline	≤ 1%	0-5	Europe, Australia

After references 2, 3, 4, 45, 46, 82, 83, 84, 85.

Epidemiology of resistance

Average estimates of primary antimicrobial resistance rates of *H. pylori* in various geographic areas or in different population groups are shown in Table II.

Resistance to macrolides

The prevalence of resistance to macrolides varies from country to country and overall seems to parallel the use of this antibiotic class for the treatment of other infections, especially respiratory tract infections. As a rule, no significant resistance rates are observed in most north European countries where the consumption of macrolide agents has always been low (34,35,36,37,38). On the other hand, a marked increase in resistance has mainly been observed in countries such as Belgium and Spain where the newer macrolides were only recently introduced (6,7,39). In Belgium, for instance, the primary resistance rate rose from 2.2% in 1990 to 11% in 1996 (6) and up to 17% in 1997 (Glupczynski, unpublished observations). In other countries, such as France where macrolides were introduced earlier on the market (i.e. in the early 80's) the resistance rates have remained stable in the range of 10-15% despite long-standing consumption (15,40). A resistance rate of 17% to clarithromycin was also reported in one study of *H. pylori* strains exclusively isolated from children in Poland (41). This high rate of clarithromycin resistance was found to parallel the high rate of macrolide prescription to children for the treatment of respiratory infection in the same country. An overview based on 1996-1997 data in Europe is presented in figure 1 while the trends of macrolide resistance in several countries where figures are available are reported in Table III. These data suggest a trend towards increased macrolide resistance in several countries. It is actually not known whether these increasing resistance rates truly reflect a higher consumption of macrolides for the specific

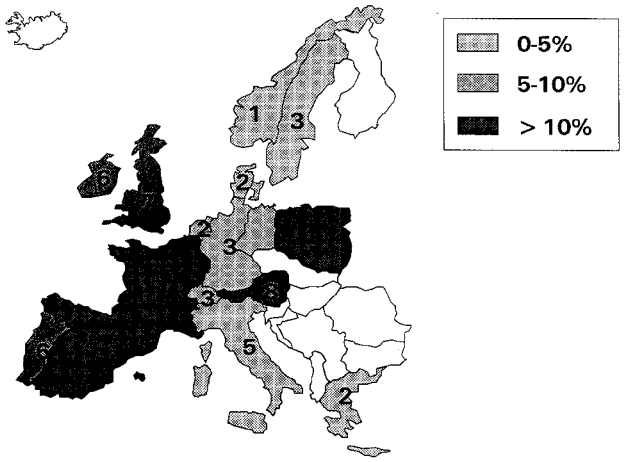

Fig. 1. — Prevalence of *H. pylori* resistance to macrolides in Western Europe (1996-1997).

Table III. — **Evolution of primary resistance to clarithromycin in *H. pylori* in different countries (1991-1994 vs 1995-1997)**

Country	1991-1994	1995-1997	p	Reference
France	9.9%	11.5%	ns	(15)
Belgium	4.8%	11.0%	0.03	(6)
Ireland	5.3%	8.6%	ns	(42)
Spain	0	12%	< 0.05	(7,39)
Unites States	3.8%	12.6%	0.024	(43)
Portugal	5.6%	8.4%	< 0.05	(44)

treatment of *H. pylori* infection. Possible selection bias may indeed have accounted for an overestimation of resistance since in many studies the number of isolates tested did originate from single hospital-based endoscopy centres. Likewise it is also possible that the indications for obtaining *H. pylori* culture and antibiotic susceptibility testing may have changed over the last years (i.e. more culture performed in *H. pylori*-infected patients with previous unsuccessful treatment attempts). In any cases, it appears now mandatory to develop

a local epidemiological surveillance of *H. pylori* resistance to macrolides.

Resistance to nitroimidazoles

A marked difference has usually been found between the rate of resistance to nitroimidazoles in developed and developing countries. The fact that metronidazole is very commonly used in tropical countries for the treatment of parasitic infections (i.e. amebiasis or giardiasis) probably accounts as a major factor for these large differences in resistance rates between industrialized an developed countries. In some countries, such as Zaire, Burkina Faso or Colombia, resistance rates can be as high as 80-90% (3,4,45).

In developed countries, the rate of resistance ranged from less than 10% to 50% in a European multicenter study in 1991, with an average figure of 28% (46). The cause of this resistance may also be linked to the use of these compounds for the treatment of genital infection, especially trichomoniasis and, therefore, strains isolated from young or middle aged women have been found more frequently to be resistant than strains isolated from men. As for the macrolides, a trend towards increased resistance has been observed both in some developed and developing countries (Table IV).

Table IV. — **Evolution of primary resistance to metronidazole in *H. pylori* in different countries (1991-1994 vs 1995-1997)**

Country	1991-1994	1995-1997	p	Reference
The Netherlands	7.3%	31.6%	< 0.0001	(8)
Belgium	29.0%	32.7%	ns	(6)
Ireland	31.8	46.3%	< 0.05	(42)
Spain	9.0%	21.6%	< 0.05	(7,39)
Unites States	40.2%	69.8%	< 0.0001	(45)
Portugal	23.0%	26.1%	ns	(44)
Singapore	45.2%	81.7%	< 0.0001	(47)
Hong-Kong	22.0%	73.2%	< 0.001	(9)

Again, it is uncertain whether this trend really reflects an increased use of these compounds to treat *H. pylori* infection. With regard to metronidazole, it is also important to stress the fact that large variations of *in vitro* susceptibility result may be observed with metronidazole depending on the methodology used, and that some techniques such as the E-test may overestimate the resistance rates to metronidazole by 10-20% (48). In one study, only metronidazole MICs determined by agar dilution, and not E-test, did correlate well with the clinical outcome in clinical trials (49).

Resistance to other antibiotics

There have been very few surveillance studies concerning resistance of *H. pylori* to classes of antibiotics other than the nitroimidazoles or the macrolides. Resistance to tetracycline has been recently detected in Australia and it has also been anecdotally reported in the United Kingdom and in Italy with a frequency of less than 5% (27,28). Tetracycline has been used rather extensively in the past as part of the two-week triple bismuth therapy that was recommended in 1990. However, this regimen is less frequently prescribed nowadays because it is superseded by the one week triple drug regimens including a proton pump inhibitor and two antibiotics (among amoxicillin, metronidazole and clarithromycin) so that resistance to this drug will probably not lead to major clinical consequences.

Resistance to fluoroquinolones has been rarely encountered with a frequency of occurrence of about 1% (28,41). This low resistance rate can probably be explained by the fact that fluoroquinolones have mostly been used to treat severe infections in the setting of hospital infection and not in the community as is the case for macrolides.

When should antimicrobial susceptibility testing be performed ?

Although the main interest of culture is to perform susceptibility tests to antimicrobial agents it cannot be recommended for routine evaluation of *H. pylori* because of the restricted availability of specialized laboratory facilities and the many potential errors involved leading to false-negative results.

On the evidence available, it seems acceptable to suggest that where the prevalence of resistance to metronidazole and/or clarithromycin is low (e.g. less than 30% resistance to metronidazole and less than 10-15% resistance to clarithromycin), routine pre-susceptibility testing would not be required in individual patients, and an initial treatment could be prescribed on a probabilistic approach based on epidemiological data of resistance at national, or better at regional level (3,4,50). If the patient has already been treated and *H. pylori* was not eradicated, they may be retreated using a regimen avoiding antibiotics used previously to which the bacterium may be resistant (e.g. if the initial antimicrobials given were clarithromycin and amoxicillin, metronidazole should be given ; if the initial antimicrobials given were metronidazole and amoxicillin, clarithromycin can be used) (51). Alternatively, culture and susceptibility testing could be used after initial treatment failure to tailor the choice of the appropriate antimicrobial therapy. Culture should be strongly recommended after a second treatment failure since in such cases, development of resistance is highly probable. Likewise, it should also be favored in patients from a geographic area or of an ethnic origin with higher likelihood of antimicrobial resistance. Actually, most centres do not perform *H. pylori* culture on a routine basis but rather recommend culture testing in selected situations were resistance is more likely to be encountered (e.g. after treatment failure). This is fraught with the risk that the resistance data gathered at many places might not reflect the background resistance rates in the community over time. It is thus deemed very

important to promote the development of national reference laboratories in order to get a correct estimation of the primary resistance rates and to follow its evolution. Such centres could also be very useful to assist routine laboratories where culture is difficult to perform.

Which susceptibility testing methods to use ?

The methods that can be used can be divided into dilution methods and diffusion methods. The dilution susceptibility testing methods can be performed either in agar or in broth and yield a quantitative result. The antimicrobial agents are tested as two-fold serial dilutions of various concentrations, depending on the agents tested and the MICs obtained are expressed in μg/ml (or in mg/l).

Although no standardized method is currently available for testing susceptibility of *H. pylori* to antibiotics, the agar dilution method is usually viewed as the most reliable technique against which all other methods should be evaluated to test their accuracy. This method is however fastidious, time consuming and it is not suited for routine use in the laboratory.

The importance of the choice of the medium (Mueller-Hinton, Iso-Sensitest, Brucella, Charcoal ...) (35), the supplementation (5-10% sheep or horse blood) (35), the size of the bacterial inoculum to be tested (10^6 to 10^9 CFU/ml) (52,53) as well as the duration of incubation (2 to 5 days) (52,53) on the susceptibility test result have each been emphasized by several investigators. Several studies are currently ongoing both in Europe and in the US in order to evaluate the relative importance of these different parameters and to propose specific guidelines for *H. pylori* susceptibility testing that can be followed and applied elsewhere.

Methods that are most currently used to test the susceptibility of *H. pylori* strains to antibiotics include the disk diffusion method (38,48,53), the breakpoint susceptibility testing (54) (a simplified version of the agar dilution method using only one or two critical antibiotic concentrations as breakpoints for resistance) and the E-test (28,48,55,56) (a quantitative variant of the disk diffusion method which yields quantitative MIC results).

Several studies have shown excellent correlations between the results obtained with these different testing methods and those achieved by the agar dilution methods, for most antibiotics including clarithromycin, amoxicillin and tetracyclines (28,48,55,56). Less good agreement was however found between E-test, disk diffusion and agar or broth dilution for the detection of resistance to metronidazole (28,48). This can probably be explained by the fact that there is a continuous distribution in the MICs of metronidazole so that the value at which the cut-off will be set or the occurrence of slight variations in the technical testing conditions may influence the final result of the test. On the other hand, when there is a clear bimodal population distribution of the MICs with a large gap between resistant and susceptible strains as is the case for clarithromycin (MICs $\leq$ 0.03 μg/ml for susceptible strains and $\geq$ 4 μg/ml for resistant isolates), all techniques seem acceptable to detect resistance (3,15).

In summary, until a standard method is formally defined and accepted, the use of the agar dilution method should be recommended for clinical trials. When testing isolates on an individual basis, all techniques, including the disk diffusion test can be used for macrolides while the breakpoint method (8 μg/ml) is to be preferred for metronidazole, and the E-test for amoxicillin.

Clinical relevance of resistance

Resistance to a given antibiotic, which is present in *H. pylori* prior to any specific treatment, is referred to as primary resistance. Such resistance is however likely to be the consequence of previous treatment with the antibiotic considered for other infections not related to *H. pylori* eventhough in most instances, the patients have no recollection of having previously taken the drug. Secondary or acquired resistance usually relates to resistance, which develops after failure of previous *H. pylori* eradication therapy. Both types of resistance can lead to a treatment failure. It is important to keep in mind that all types of resistances that occur in *H. pylori* are acquired by mutations and can be prevented by the administration of a second antibiotic and by ensuring that sufficient concentrations are achieved at the site of infection. It is thus of paramount importance always to prescribe two antibiotics and to explain carefully to the patient the importance of taking all medications together without interruption until the end of the therapy. Compliance to treatment is a key factor in preventing the occurrence of resistant strains.

To date primary and secondary resistances have mostly been reported to occur with the nitroimidazoles and with the macrolides.

Metronidazole

In most studies where a standard bismuth therapy was used, lower cure rates were usually found when the infecting *H. pylori* isolates turned out to be resistant to metronidazole (Table V). Triple therapies associating amoxicillin, metronidazole and a proton pump inhibitor have also found to be consistently less effective in patients with metronidazole-resistant strains than in subjects infected with metronidazole-susceptible ones (42-77% cure rates vs. 76-96%, respectively) (Table V). In a clinical trial using amoxicillin, metronidazole and lansoprazole for 10 days (49), a pre-treatment MIC value of metronidazole $>$ 8 μg/ml (determined by agar dilution) proved clinically relevant and was associated with a 50% cure rate vs. 76% for those strains with MICs less than 8 μg/ml. On the other hand, the

Table V. — **Clinical relevance of metronidazole resistance on the efficacy of various *H. pylori* eradication regimens**

Name of first author (Ref.)	Treatment regimen*	Eradication (*Metro-S*)	Eradication (*Metro-R*)
Triple standard therapy with bismuth salts (7-14 days)			
Logan (57)	CBS-Amox-Metro 7 d	40/43 (93%)	4/21 (19%)
Burette (58)	CBS-Amox-Metro 10 d	29/30 (90%)	17/27 (63%)
Rautelin (59)	CBS-Amox-Metro 14 d	(91%)	(63%)
Noach (60)	CBS-Tetra-Metro 7 d	47/49 (96%)	11/29 (38%)
Lerang (61)	BSN-Tetra-Metro 10 d	116/128 (91%)	65/83 (78%)
Low-dose triple therapy with an antisecretory drug (7 days)			
Bouchard (49)	Lanso-Amox-Metro 10 d	59/77 (76%)	9/18 (50%)
Lerang (62)	Ome-Amox-Metro 10 d	48/50 (96%)	17/22 (77%)
Thijs (63)	Ome-Amox-Metro 7 d	(95%)	(69%)
Miyaji (64)	Lanso-Amox-Metro 14 d	14/16 (87%)	5/12 (42%)
Harris (65)	Lanso-Clar-Metro 7 d	22/24 (92%)	12/16 (75%)
Moayyedi (66)	Ome-Clari-Tini 7 d	62/69 (90%)	42/45 (93%)
Lind (67)	Ome-Clari-Metro 7 d	(95%)	(76%)
Georgopoulos (68)	Ome-Clari-Metro 7 d	20/20 (100%)	7/12 (57%)
Buckley (69)	Ome-Clari-Metro 7 d	55/56 (98%)	16/28 (57%)
Lerang (62)	Ome-Clari-Metro 10 d	45/48 (94%)	17/18 (94%)
Peitz (70)	Ome-Clari-Metro 7 d	71/76 (94%)	52/59 (88%)
Quadruple therapy including a triple standard therapy plus an antisecretory drug			
Van der Hulst (71)	CBS-Tetra-Metro-Ome 7 d	42/42 (96%)	32/39 (82%)
Graham (72)	BSS-Tetra-Metro-Lanso 10 d**	24/25 (96%)	7/17 (41.2%)

*CBS : colloidal bismuth subcitrate ; BSN : bismuth subnitrate ; Metro : metronidazole ; Tini : tinidazole ; Amox : amoxicillin ; Ome : omeprazole ; Lanso : lansoprazole ; S : susceptible ; R : resistant.

**Twice-a-day quadruple therapy.

impact of metronidazole resistance on the outcome of low-dose short-term (1 week) triple therapies including metronidazole and clarithromycin has shown great variations, with *H. pylori* cure rates ranging from 57 to 94%, as opposed to consistently > 90% in patients with metronidazole-sensitive strains (62,66,67,68,69,70). Various investigators (62,66,70) found identical cure rates in patients infected with metronidazole-susceptible or -resistant strains. In one trial, Moayyedi *et al.* (66) performed disc diffusion and E-test studies for determination of the MICs before treatment and found similar cure rates in patients with susceptible or resistant strains (57% vs 56%, respectively) when a cut-off value of 8 μg/ml was used to define resistance. However, after having redefined the breakpoint for resistance as MIC > 32 μg/ml, eradication rates were 70% in sensitive but only 30% in resistant strains. This observation also suggests that the breakpoint to define metronidazole resistance should perhaps also be different, depending on the antibiotic combination used On the whole, it is thus probable that differences in the methodology used for susceptibility testing as well as in the breakpoint used to categorize *H. pylori* isolates as resistant may partly account for the discrepancies observed between studies with regard to the clinical relevance of metronidazole resistance. As mentioned earlier, it is possible that the E-test may overestimate the true rate of metronidazole resistance (48). Moreover correlations between different susceptibility testing methods have yielded suboptimal results for metronidazole, with only 9 of 16 E-test MIC values being in the range of ± 1 dilution of those obtained by the agar dilution (56).

Clarithromycin

Because of the lower prevalence of *H. pylori* resistance to macrolides there are relatively fewer data concerning its clinical relevance. However, in clinical trials where susceptibility testing data are available, a good correlation was usually found between clarithromycin MIC results and the treatment outcome. In dual therapies where clarithromycin was used as the only antibiotic with an antisecretory drug for 2 weeks, the rate of eradication was only in the range of 0-25% (possibly depending on clarithromycin dosage) in patients infected with clarithromycin-resistant strains vs. 60-65% for those with clarithromycin susceptible isolates (73,74,75,79). Development of resistance was usually found to occur in at least 60% of the treatment failures

Table VI. — **Clinical relevance of clarithromycin resistance on the efficacy of various *H. pylori* eradication regimens**

Name of first author (Ref.)	Treatment regimen*	Eradication (*Clari-S*)	Eradication (*Clari-R*)
Dual therapy			
Burette (73)	Ome-Clari 14 d	10/16 (62.5%)	0/2
Cayla (74)	Ome/Lanso-Clari 14 d	20/31 (64.5%)	1/4 (25%)
Schütze (75)	Rani-Clari 12 d	21/34 (62%)	1/5 (20%)
Miyaji (64)	Lanso-Clari 14 d	7/11 (64%)	0/12
Triple therapy			
Cayla (74)	Ome-Clari ± Amox 14 d	77/93 (83%)	3/10 (30%)
Lamouliatte (76)	Lanso-Clari-Amox 14 d	42/43 (98%)	1/4 (25%)
Burette (77)	Ome-Clari-Amox 12 d	30/34 (88%)	1/2 (50%)
Burette (78)	Lanso-Clari-Amox 10 j	73/76 (96%)	4/8 (50%)
Würzer (79)	Ome-Clari-Amox 10 d	92/100 (92%)	3/6 (50%)

*Clari : clarithromycin ; Metro : metronidazole ; Amox : amoxicillin ; Ome : omeprazole ; Lanso : lansoprazole ; Rani : ranitidine ; S : susceptible ; R : resistant.

in patients initially infected with a susceptible strain (69,74,80). The use of triple therapy combining amoxicillin plus clarithromycin and an antisecretory drug has proved overall very effective with eradication rates of > 90% in patients harboring clarithromycin-susceptible isolates. However, in patients with clarithromycin-resistant strains, the cure rates averaged at best 30-50%, although it must be stressed that very small number of resistant isolates were encountered in all the studies (74,77,78,79).

Simultaneous clarithromycin and metronidazole resistance has also been recently reported to occur most likely as a consequence of previous treatment failure with regimens in which these antibiotics were included. In one center in Brussels, the frequency of double resistant *H. pylori* isolates was found having significantly increased over time from 0% in 1991 up to 4% in 1996 (6). It is important to stress that none of the current triple drug regimens are effective against these "multi-resistant" strains. The quadruple therapy consisting in a standard triple therapy (bismuth salts-tetracycline-metronidazole) plus a proton pump inhibitor (81) may possibly constitute a good alternative but it needs to be evaluated specifically in this setting.

Conclusion

Development of drug resistance in *H. pylori* to the nitroimidazoles and to the macrolides accounts as the first cause of failure with current eradication regimens. Moreover, failed eradication frequently leads to the development of secondary resistance to metronidazole and to clarithromycin. While complete eradication of *H. pylori*-associated gastroduodenal pathologies appears as a possible and desirable objective, it can be anticipated that an increase in the resistance rates as well as the development of multi-drug resistant organisms will endanger the efficacy of our current treatment regimens in the future. In order to curb such developments the following recommendations can be made : 1) avoid monotherapy and always recommend the use of a compound for which resistance may occur in association with a second antibiotic ; 2) favor the use of simple treatment regimens and stress the importance of compliance which is a both a key factor for treatment success and also for preventing the occurrence of resistant strains ; and 3) institute national and regional surveillance programs to follow the evolution of *H. pylori* resistance and to better adapt treatment regimens to changes in resistance patterns.

Progresses should also be accomplished in the field of susceptibility testing, e.g. standardization of the susceptibility testing procedures and development of rapid molecular tests, in order to improve the detection of resistance and to allow the monitoring of antimicrobial resistance in *H. pylori* worldwide. Further research should also be directed towards the development by the pharmaceutical industry of new active compounds against which *H. pylori* will not develop resistance. At last, knowledge of the entire sequence of the *H. pylori* genome should render possible in a near future the development of novel inhibitory or bactericidal drugs that would specifically target vital function of *H. pylori* strains.

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